CHARACTERIZATION AND IDENTIFICATION OF AN UNKNOWN COMPOUND ASSOCIATED WITH METABOLIC ACIDOSIS IN DIARRHEIC MAMMALS

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Division of Nutrition and Dietetics

College of Pharmacy and Nutrition

University of Saskatchewan

Saskatoon, Saskatchewan

By

Wade Barabash

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

DISCLAIMER

Reference in this thesis to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Division of Nutrition and Dietetics
College of Pharmacy and Nutrition
University of Saskatchewan
110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada

OR

Dean
College of Graduate Studies and Research
University of Saskatchewan
107 Administration Place
Saskatoon, Saskatchewan S7N 5A2
Canada

ABSTRACT

Organic acids, including L- and D-lactate, explain most but not the entire elevated anion gap in diarrhea-associated metabolic acidosis. Also, D-lactate has been implicated in the neurological symptoms associated with this condition. Less-common organic compounds may influence the anion gap and neurological symptoms. This research aimed to characterize and attempt to identify a previously unidentified compound, Compound X, first noted in diarrheic acidotic calves with elevated anion gap (Omole, 1999).

High performance liquid chromatography (HPLC) was used to measure Compound X in biological fluids from diarrheic and healthy calves; diarrheic piglets, foals, and human infants; and calves experimentally infused with saline or acid. Attempts were made to identify Compound X using HPLC with tandem and Fourier-transform mass spectrometry.

Compound X was significantly higher in diarrheic calf serum ($p<0.001$) and lower in feces ($p<0.001$) and rumen fluid ($p<0.001$) than those fluids from healthy calves. Compound X in serum from acid-infused calves (median peak area ratio = 1.5 – 1.9) was lower than that of diarrheic calves (median = 4.8) and only slightly greater than that of healthy calves (median = 1.2). Serum Compound X correlated with serum D-lactate in diarrheic and healthy calves combined; however, no such correlation was observed in acid-infused calves. Conversely, a relationship between Compound X and neurological disturbance was present in acid-infused calves, but not in diarrheic calves. In other species, Compound X was highest in diarrheic infants and lowest in diarrheic piglets. Although mass spectrometry and database library searches revealed several compounds as putative matches for Compound X, none of the compounds made sense within the context of acidosis and mammalian biological fluids. Therefore, the identity of Compound X remains unknown.

Compound X has been established as a ubiquitous compound(s) present in the biological fluids of mammals. Compound X may be a normal intestinal compound or bacterial metabolite that crosses the intestinal epithelium during diarrhea. In spite of this, Compound X was associated with the neurological manifestations of D-lactic acidosis. Compound X`s identity was not determined, and some reasons for this and future directions are discussed.

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge my graduate studies supervisor, Dr. Gordon Zello, for his guidance, sharing of his wisdom, and his patience. I would also like to acknowledge the members of my advisory committee, Dr. Denis Lehotay, Dr. Susan Whiting, and Dr. Jonathan Naylor, for their expert direction and support throughout the course of my studies. I am grateful to Phenomenome Discoveries Incorporated (Saskatoon, Canada) for performing mass spectrometry of my samples and answering all of my related questions. I am thankful to Wayne Hunter and Diana Schneider (RUH Metabolic Diseases Lab, Saskatoon) for performing gas chromatography/mass spectrometry of my samples. I am also grateful to previous graduate students, Olutosin Omole, Julia Ewaschuk, and Saman Abeysekara, for the work they performed to obtain the samples analyzed for my project. I am thankful to my fellow graduate students, Dawn Ciona, Abdulla Alemmari, Sam Abeysekara, Jennifer Adolphe and others whom I have known throughout my program, for their support and friendship.

I gratefully acknowledge the financial support provided by the Natural Sciences and Engineering Research Council of Canada Graduate Scholarship, the University of Saskatchewan Dean's Scholarship, and the University of Saskatchewan Hope Hunt Scholarship.

DEDICATION

This thesis is dedicated to my wife, Audra, and our children, who inspired and supported me throughout my graduate studies program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	degrees Celsius
APAR	average peak area ratio
AG	anion gap
BVDV	bovine viral diarrhea virus
Cl^-	chloride ion
CNS	central nervous system
CO_2	carbon dioxide
CRC	Chemical Rubber Company
CSF	cerebrospinal fluid
Da	Daltons
E. coli	*Escherichia coli*
ESI	electrospray ionization
ETEC	Enterotoxigenic *Escherichia coli*
FTMS	Fourier-transform mass spectrometry
FWHM	full-width half-height maximum
GABA	gamma-aminobutyric acid
GC/MS	gas chromatography and mass spectrometry
H_2CO_3	carbonic acid
HCl	hydrochloric acid
HCO_3^-	bicarbonate
HPLC	high performance liquid chromatography
K^+	potassium ion
LC/MS/MS	liquid chromatography with tandem mass spectrometry
m	metre
m/z	mass-to-charge ratio
µL	microlitre
mL	millilitre
µm	micrometer
mM	millimolar
MS/MS	tandem mass spectrometry
MWCO	molecular weight cutoff
n	sample size
Na^+	sodium ion
NH_4^+	ammonium ion
nm	nanometre
NMR	nuclear magnetic resonance
p	p-value or probability
Pa_{CO_2}	partial pressure of carbon dioxide in arterial blood
PAR	peak area ratio
ppm	parts per million
RT	retention time
SD	standard deviation
spp.	species
u	unified atomic mass units

USA	United States of America
USDA	United States Department of Agriculture
UV	ultraviolet
WCVM	Western College of Veterinary Medicine
WHO	World Health Organization
x g	multiplied by the force of gravity, centrifugal force units

1. INTRODUCTION

1.1. Rationale

Diarrhea is a significant health problem affecting humans and domestic animals worldwide. The World Health Organization has reported that approximately 2.5 billion cases of diarrhea occur in children less than five years of age each year, causing 1.5 million deaths in this population (The United Nations Children's Fund (UNICEF)/World Health Organization (WHO), 2009). The primary sequelae of diarrhea include dehydration and metabolic acidosis, almost regardless of diarrheal etiology (Grove-White & White, 1993). Diarrhea-associated metabolic acidosis was historically associated with L-lactate production; however, D-lactate has been reported as a significant contributor to metabolic acidosis in diarrheic calves (Ewaschuk, Naylor, Chirino-Trejo, & Zello, 2004; Ewaschuk, Naylor, Palmer, Whiting, & Zello, 2004; Lorenz & Vogt, 2006; Omole, Nappert, Naylor, & Zello, 2001) and humans with short bowel syndrome (Azhar & Beach, 2002; Zhang, Jiang, Jiang, Cao, & Li, 2003). One way of classifying metabolic acidoses uses the anion gap, which is the difference between commonly measured plasma cations and commonly measured plasma anions (Ewaschuk, Naylor, & Zello, 2003). Thus, the anion gap represents the unmeasured anions present in a biological fluid, such as serum or urine (Ewaschuk et al., 2003). In diarrhea, D-and L-lactate explain some, but not all, of the increase in anion gap.

In 1999, Omole (Omole, 1999) used high performance liquid chromatography (HPLC) to measure D- and L-lactic acids, as well as pyruvic and acetic acids, in diarrheic and healthy calf serum. He observed an unidentified peak, termed Compound X, on the chromatograms and found Compound X to be higher in the diarrheic group and to correlate with the anion gap (Omole, 1999). It was suggested that Compound X was an organic acid, or a compound with properties similar to organic acids, that contributed to the anion gap (Omole, 1999).

Ewaschuck (Ewaschuk, 2004) measured D- and L-lactate in diarrheic calves, as well as three diarrheic monogastric species – human infants, piglets, and foals. Compound X was not studied, however diarrheic human infants presented with elevated anion gap that was not attributable to increased D- or L-lactate (Ewaschuk, 2004). Ewaschuk (Ewaschuk, 2004) concluded that another organic acid was responsible for the elevated anion gap, although there was no mention of Compound X (Ewaschuk, 2004).

Metabolic acidosis has also been associated with effects on the central nervous system. In calves, metabolic acidosis has been correlated with depression scores (Nakagawa, Suzuki, &

Taguchi, 2007). Some research has suggested a role for D-lactate in acidosis-related neurologic disturbance (Abeysekara, Naylor, Wassef, Isak, & Zello, 2007; Lorenz & Vogt, 2006). In humans, D-lactic acidosis has been associated with neurological symptoms, including slurred speech, ataxia, and severe lethargy (Uchida et al., 2004; Zhang, 2000), as well as memory loss and disorientation (Narula, El Shafei, Ramaiah, & Schmitz, 2000). One hypothesis regarding the mechanism of neurologic disturbance in D-lactic acidosis is that unknown toxic compounds produced along with D-lactate, and not D-lactate per se, are responsible for the observed signs (Uribarri, Oh, & Carroll, 1998).

Diarrhea and its sequelae have devastating consequences to human and animal welfare, along with significant economic effects to the health care system and agricultural sector. Diarrhea-associated metabolic acidosis and the associated neurological manifestations remain incompletely understood. There are potentially unknown metabolites, produced by the body or by intestinal bacteria, that contribute to the elevated anion gap in diarrheic animals (Omole, 1999) and human infants (Ewaschuk, 2004), and the mechanism of neurological manifestations from metabolic acidosis are unknown. For these reasons, unidentified compounds associated with diarrhea and metabolic acidosis must be examined to elucidate their potentially significant roles in these conditions.

1.2. Objectives

This study aimed to characterize the role of the unknown Compound X, first noted by Omole (Omole, 1999), in diarrhea-associated metabolic acidosis. The first objective was to determine if Compound X was related to diarrheal illness, D-lactic acidosis, or acidosis-related neurological disturbance. The second objective was to determine the origin of Compound X. The third objective was to determine whether or not Compound X is present in other diarrheic animals. The final objective was to determine the identity of Compound X.

2. LITERATURE REVIEW

2.1 Diarrhea

Diarrhea is a significant health problem affecting humans and domestic animals worldwide. The World Health Organization has reported that approximately 2.5 billion cases of diarrhea occur in children under five years of age each year, causing 1.5 million deaths in this population (The United Nations Children's Fund (UNICEF)/World Health Organization (WHO), 2009). In cattle, although mortality rates from diarrhea have declined, diarrhea remains the most common neonatal ailment and is responsible for significant economic loss to livestock producers (Ewaschuk, Naylor, Chirino-Trejo et al., 2004).

Diarrhea can be caused by osmotic, secretory, exudative, or motility factors, operating alone or in combination (Field, 2003). Osmotic diarrhea occurs when ingested solutes remain unabsorbed by the intestinal epithelium, drawing water and ions into the intestinal lumen in excess of the colon's capacity for reabsorption (Field, 2003). Secretory diarrhea can be caused by bacterial enterotoxins, bile acids, hormones, or inflammatory mediators that create aberrant ion transport in which electrolytes and water move from the intestinal epithelium into the lumen and overwhelm the colon's absorptive capacity (Field, 2003; Sellin, 2001). Exudative diarrhea refers to the leakage of water, electrolytes, mucus, and protein from intestinal lymph and blood vessels into the intestinal lumen secondary to intestinal epithelial impairment, such as disrupted tight junctions (Field, 2003). Intestinal motility-related diarrhea can result from increased motility, as in hyperthyroidism, as well as decreased motility, which can lead to diarrhea due to intestinal bacterial overgrowth (Field, 2003; Sellin, 2001).

Diarrhea of a secretory and osmotic nature, often due to rotavirus infection or enterotoxigenic *Escherichia coli* (ETEC) infection, occurs more frequently and causes more mortality than the other types of diarrhea in humans (Baldi, Bianco, Nardone, Pilotto, & Zamparo, 2009; Widdowson, 2005), calves (Acres, 1977; Foster, 2009), and other mammals (Imagawa et al., 1991; Moeser, 2007a). The primary sequelae of diarrhea include dehydration and metabolic acidosis, almost regardless of diarrheal etiology (Grove-White & White, 1993).

2.1.1 Diarrhea in humans

Diarrhea is a leading cause of morbidity and mortality in developing countries, and while worldwide mortality from diarrheal disease has declined over the past half-century, morbidity due to diarrhea has remained relatively unchanged (Kosek, 2003; Parashar, Bresee, & Glass, 2003). In developing countries, it has been estimated that children under five years of age average three diarrheic episodes per year, with diarrhea being responsible for 19.5 – 21% of all deaths in this age group (Kosek, 2003). Estimates are that Americans experience 0.7 diarrheic episodes per person, equivalent to a total of 200 million episodes, per year (Herikstad, 2002). The prevalence of diarrheic illness in Canada has been estimated to be 0.99 episodes per person per year, with 20.4% of people seeking medical attention for diarrheic illness (Scallan et al., 2005). The importance of diarrheic illness is underscored by the fact that Canada's population is presently greater than 30 million.

Diarrhea and malnutrition interact, particularly in developing countries, in a perpetual cycle (Tomkins & Watson, 1989). Diarrhea causes a decline in nutritional status, more so with persistent diarrhea than acute diarrhea, and poor nutritional status can subsequently increase the duration of diarrheic episodes (Tomkins & Watson, 1989). The effects of diarrhea on nutritional status can be long-term, and a recent meta-analysis reported a dose-response relationship between duration of diarrheal illness and degree of linear growth stunting in children (Checkley, 2008). The mechanisms by which diarrhea and diarrhea-associated infections produce these effects include reduced intake, reduced nutrient absorption, increased nutritional requirements, and increased nutrient losses (Tomkins & Watson, 1989).

In addition to the human cost of diarrheic illness, diarrhea carries a financial cost to sufferers, business, and health care. In the USA, Herikstad et al. (Herikstad, 2002) reported an overall diarrhea prevalence of 11%, with the prevalence being 10% in children less than five years of age. While Herikstad et al. (Herikstad, 2002) did not determine the economic impact of diarrheal illness, the annual economic impact of human gastroenteritis in Australia, whose population is one-tenth that of the USA, has been estimated to be more than $340 million (Hellard, 2003). Diarrheal illness prevalence in Canada slightly exceeds that of Australia, and Canadians seek medical attention at rates comparable to Australians during diarrheal illness (Scallan et al., 2005). Canada's population in mid-2009 was approximately 50% larger than Australia's population (World population data sheet, 2009), conceivably suggesting that the

economic burden of human diarrheic illness in Canada exceeds Australia's estimated $340 million annual cost.

2.1.2 Diarrhea in other animals

Diarrhea is one of the most common reasons that animals present to veterinary clinics (Brown, 2008; Marks, 2003). It is problematic in both small animals, such as dogs (Marks, 2003; Yesilbag, 2007) and cats (Zoran, 2008), and large animals, such as horses (Imagawa et al., 1991), elk (Keppie, 2005), pigs (Holland, 1990; Moeser, 2007b), and cattle (Foster, 2009; Gow, 2005; Holland, 1990; Ott, 1993). Diarrhea produces significant morbidity and mortality in foals (Magdesian, 2005) and, additionally, is responsible for poor growth in piglets, causing substantial economic loss to the swine industry (Moeser, 2007b). In cattle, particularly neonatal calves, diarrhea is one of the most important causes of illness and death, resulting in significant economic loss to producers (Foster, 2009).

2.1.2.1 Diarrhea in calves

The cattle industry is a major agricultural and economic sector in North America. In 2006, Canadian cattle sales totaled $6.5 billion and were the largest source of farm cash receipts (Beef Information Centre, 2007). As of January 1, 2009, the North American cattle inventory was 108 million head, and the 2008 retail value of the United States beef industry was $76 billion (United States Department of Agriculture Economic Research Service, 2009).

Bovine viral diarrhea virus (BVDV), a common infectious agent in calves (Taylor, 1997), causes mucosal disease and is a major animal welfare concern, as it leads to considerable animal distress in both young and older cattle (Valle, 2005). The virus, transmitted via pregnant dams to fetuses, results in offspring who are immunotolerant persistent shedders of the virus (Goens, 2002). BVDV has been associated with reduced production of milk, fat, and protein by Canadian dairy cows (Tiwari et al., 2007) and has been estimated to increase the hazard of culling of Canadian dairy cows by 1.86 times (Tiwari et al., 2005). However, BVDV affects cattle across the lifespan and is not specific to the neonatal period.

Neonatal calf diarrhea, most often of an infectious nature (Foster, 2009) has been estimated to be responsible for 49.4 – 57.2 % of preweaned dairy heifer deaths in the USA, depending on the season (Ott, 1993). The United States Department of Agriculture (USDA) has

reported scours/diarrhea to be the leading cause of mortality, accounting for 56.5% of all preweaned dairy heifer deaths (USDA, 1991-2007). The USDA also determined digestive problems (diarrhea, acidosis, bloat, etc.) to be responsible for 21.2% of the 2.3 million calf deaths and 11.1% of the 1.7 million cattle deaths in 2005 (United States Department of Agriculture, 2007). The infectious culprits in neonatal calf diarrhea are most often enterotoxigenic *E. coli* (ETEC), *Cryptosporidium parvum*, coronavirus, and rotavirus (Foster, 2009).

Enterotoxigenic *E. coli* tends to cause secretory diarrhea in the first four days of life by colonizing the gastrointestinal tract before normal commensal bacteria are firmly established. *Cryptosporidium parvum* and coronavirus infection cause malabsorptive diarrhea secondary to villous atrophy, while rotavirus infection gives rise to a combination malabsorptive-secretory diarrhea (Foster, 2009). The parasitic *Cryptosporidia* species are found on nearly 60 % of American dairy farms, and the most pathogenic strain, *Cryptosporidium parvum*, tends to infect younger, and particularly pre-weaned, calves at a much higher rate than older calves, heifers, and milking cows (Fayer, 2007). Coronavirus and rotavirus typically infect calves younger than three weeks of age, and most calves demonstrate exposure to these viruses, as evidenced by antibody titers (Foster, 2009). In view of the widespread and detrimental nature of diarrheal illness, an improved understanding of the factors associated with calf diarrheic illness will lead to improvements in animal welfare and reduce economic loss associated with morbidity and mortality of diarrheic calves.

2.2 Metabolic Acidosis

Metabolic acidosis, which is common in diarrhea, is the accumulation of hydrogen ions in the blood caused by the loss of bicarbonate (base) from, or addition of acid to, the blood (Ewaschuk, Zello, Naylor, & Brocks, 2002). Acidosis is a disorder that results in acidemia, which is defined as an arterial blood pH below 7.35 (Ayers & Warrington, 2008).

2.2.1 Regulation of pH

Acids are compounds capable of donating protons (H^+), while bases are compounds capable of accepting H^+ (Morris & Low, 2008a). Acidity is expressed using a logarithmic scale (Morris & Low, 2008a) as $pH = -\log_{10}[H^+]$…….(2.1)

Normal human blood pH is between 7.38 and 7.42 (Gauthier, 2002) and is determined by the ratio of bicarbonate (HCO_3^-) to carbon dioxide (CO_2) in the blood (Ayers & Warrington, 2008). The body normally maintains this pH range with a combination of chemical buffering, respiratory, and renal mechanisms (Edwards, 2008).

The Henderson-Hasselbalch equation describes the metabolic and respiratory contributions to arterial pH as follows (Dubose, 2008):

$$pH = 6.1 + \log \frac{[HCO_3^-]}{Pa_{CO_2} \times 0.0301} \quad \text{.....(2.2),}$$

where Pa_{CO_2} is the arterial partial pressure of carbon dioxide. Changes in the body's acid or base production are initially counteracted by buffering systems, including carbonic acid-bicarbonate, phosphate, and plasma proteins, but when the changes persist, respiratory and renal mechanisms must compensate and correct to restore homeostasis (Clancy, 2007). Neural respiratory mechanisms regulate the blood CO_2 level by altering respiration rate, while renal mechanisms regulate the blood HCO_3^- concentration via reabsorption of filtered HCO_3^-, production of titratable acid, production of HCO_3^-, and excretion of NH_4^+ (Dubose, 2008).

Acid excretion is a renal mechanism of regulating pH. Excretion of the acid load, be it by urinary excretion or renal metabolism, by the body can be classed into three categories, namely volatile acids (i.e., CO_2/H_2CO_3), organic acids (e.g., lactate, citrate), and inorganic acids (e.g., phosphate, sulphate) (Morris & Low, 2008a). Acidoses are typically divided, based on the type of excess acid present, into respiratory acidosis and metabolic acidosis (Kellum, 2005). Respiratory acidoses are those that reduce systemic pH by increasing Pa_{CO_2}, whereas metabolic acidoses are those that decrease systemic pH by reducing HCO_3^- without changing Pa_{CO_2} (Kellum, 2005). Mixed acidoses, in which both metabolic and respiratory factors contribute to reduced pH, can occur (Kellum, 2005).

2.2.2 Anion gap

Metabolic acidoses can be classified based on the presence or absence of an elevated anion gap (Gauthier, 2002). The anion gap (AG) is defined as the difference between the measured cations and measured anions in plasma, and is represented by the following equation (Fidkowski & Helstrom, 2009):

$$AG = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]) \quad \text{.....(2.3)}$$

The normal human AG is 12 ± 2 mEq/L (LeBlond, Brown, & DeGowin, 2009). Total (i.e., measured plus unmeasured) cations must equal total anions in a biological system, and regarding AG, this principle dictates that as unmeasured anions increase in body fluids, HCO_3^- decreases and results in elevated AG (Gauthier, 2002).

The four major causes of elevated AG metabolic acidosis are lactic acidosis, ketoacidosis (common in diabetes), renal failure, and drug intoxication from salicylates, methanol, or ethylene glycol (Ayers & Warrington, 2008; Morris & Low, 2008b). The cause of normal AG metabolic acidosis is hyperchloremic in nature and commonly related to renal tubular acidosis, renal failure, or diarrhea (Ayers & Warrington, 2008; Gauthier, 2002; Morris & Low, 2008b). However, these divisions between elevated AG and normal AG acidoses are not always clear. For example, critically ill patients experiencing increased organic acid accumulation, which should create an elevated AG, sometimes present with reduced plasma proteins and phosphate (i.e., unmeasured anions), effectively lowering the AG and offsetting any AG elevation caused by organic acid accumulation (Fidkowski & Helstrom, 2009).

2.2.3 Metabolic acidosis in diarrheal illness

A primary consequence of diarrhea, metabolic acidosis is the accumulation of hydrogen ions in the blood caused by the loss of bicarbonate from, or addition of acid to, the blood (Ewaschuk et al., 2002). Historically, diarrhea-associated metabolic acidosis has been attributed to the L-isomer of lactate which is produced anaerobically by ischemic tissues during critical illness, as well as to bicarbonate loss in the feces (Ewaschuk et al., 2002). However, L-lactate is not solely responsible for the acidosis in all cases. The D-isomer of lactate has been reported as a significant contributor to metabolic acidosis in diarrheic calves (Ewaschuk, Naylor, Chirino-Trejo et al., 2004; Ewaschuk, Naylor, Palmer et al., 2004; Lorenz & Vogt, 2006; Omole et al., 2001), as well as in humans with short bowel syndrome (Azhar & Beach, 2002; Zhang et al., 2003).

Furthermore, elevated AG metabolic acidosis is sometimes not explained by either isomer of lactic acid. Ewaschuk (Ewaschuk, 2004) measured D- and L-lactate in human infants and reported a mean anion gap of 23 mmol/L (normal 12 ± 2 mmol/L), with D- and L-lactate accounting for a combined mean of 2.1 mmol/L. It was concluded that other organic acids may

contribute to the acidosis, although the contribution of elevated plasma proteins (secondary to dehydration) to the anion gap was not evaluated (Ewaschuk, 2004).

2.2.4 Neurological effects of metabolic acidosis

Metabolic acidosis has been associated with central nervous system effects. In calves, venous pH and bicarbonate values (i.e., metabolic acidosis) have been negatively correlated with depression scores (Nakagawa et al., 2007). Some research has suggested a role for D-lactic acid in acidosis-related neurologic disturbance. Lornez and Vogt (2006) (Lorenz & Vogt, 2006) observed associations between D-lactic acid levels and listless demeanor and wobbly posture in calves. Abeysekara et al. (Abeysekara et al., 2007) found strong correlations between serum or cerebrospinal fluid (CSF) D-lactic acid levels and neurologic impairment, as measured by strength of suck, ability to stand, and palpebral, menace, and tactile reflexes, in acid-infused calves.

Within intensive care settings, metabolic acidosis is common, with acidemia impairing cardiovascular function, respiratory function, and neurologic function (Gauthier, 2002). In humans with short bowel syndrome, D-lactic acidosis has been associated with a range of neurologic symptoms. Dahlquist et al. (Dahlquist, Perrault, Callaway, & Jones, 1984) reported on two adult patients who had undergone obesity treatment via jejunoileostomy and later developed D-lactic acidosis with encephalopathy consisting of confusion, slurred speech, unsteadiness, weakness, lethargy, and hostile behavior. Similar symptoms were observed in a 22-year-old man with short bowel syndrome and D-lactic acidosis (Uchida et al., 2004). D-lactic acidosis with encephalopathy has also occurred in infants and children with SBS subsequent to bowel resection (Gurevitch, 1993), and Zhang et al. (Zhang et al., 2003) reported on a 12-year-old boy with short bowel syndrome and D-lactic acidosis who exhibited recurring neurologic symptoms, including slurred speech, ataxia, and severe lethargy, over a 12-month period.

2.2.4.1 Possible mechanisms of neurological disturbance in metabolic acidosis

Neurological symptoms associated with D-lactic acidosis have often been attributed to acidosis and elevated blood D-lactate concentration, but some reports contradict these hypotheses (Petersen, 2005). Acidemia alone is not a likely cause of neurological symptoms, since acidemias from etiologies other than D-lactic acid do not cause neurological disturbance

(Abeysekara, Naylor, & Zello, 2006; Petersen, 2005). This was demonstrated by Abeysekara (Abeysekara et al., 2006), who compared the neurological effects of hydrochloric acid (HCl)-induced and L-lactic acid-induced acidemia with those of D-lactic acid-induced acidemia in calves. That study reported significant inhibitory neurological disturbance from D-lactic acidemia, but not from acidemia produced by HCl or L-lactic acid infusion (Abeysekara et al., 2006).

One hypothesis regarding the mechanism of neurologic disturbance in D-lactic acidosis is that unknown toxic compounds produced along with D-lactate, and not D-lactic acid itself, are responsible for the observed signs (Uribarri et al., 1998). One instance in which a toxin precipitates neurologic symptoms is ciguatera poisoning, a seafood-toxin illness characterized by diarrhea and neurologic disturbance, with the neurologic disturbance mechanism being related to a toxin produced by an ingested microalgae (Wong, 2008). This hypothesis is supported by evidence showing that D-lactate is in fact metabolized and excreted from the body reasonably well, which suggests that D-lactate may not be the cause of neurological impairment.

D-lactate is metabolized in mammals by D-2-hydroxyacid dehydrogenase (Tubbs, 1965), and the metabolic rate has been reported to be 75% of an infusion rate of 1.8 - 2.0 mEq/kg/h (Oh et al., 1985). D-lactate is excreted in urine, and its fractional excretion is positively correlated to concomitant L-lactate levels (Oh et al., 1985). Further, serum D-lactic acid greater than 0.5 mmol/L has been observed in asymptomatic humans with previous jejunoileal bypass surgery, although the threshold level required to trigger symptoms is unknown (Thurn, 1985). In light of the observations that humans can be asymptomatic during periods of D-lacatatemia and mammals are able to metabolize and excrete D-lactate, it is possible that other compounds are responsible for the neurologic manifestations of D-lactic acidosis.

Alternatively, D-lactate may have an indirect effect on neurologic status through interference of vital metabolic processes within the brain. Up-regulated glycolytic activity has been observed in activated neurons and astrocytes, with L-lactate being released and subsequently taken up and carried away to the bloodstream by astrocyte networks (Gandhi, 2009). It has been proposed that astrocytic L-lactate removal is necessary to maintain the higher glycolytic rate of activated brain cells (Gandhi, 2009). Since D-lactate and L-lactate compete for transport into cells via monocarboxylate transporters, D-lactate may produce neurological effects as a result of impeded L-lactate removal from brain cells. Another mechanism involving

astrocytes arose from a study on one-day-old chicks, in which D-lactic acid injected into the brain was shown to impair memory formation (Gibbs, 2008). The proposed mechanism for this involves astrocytes being vital for this brain function and D-lactate inhibiting pyruvate uptake and metabolism in astrocyte mitochondria (Gibbs, 2008).

2.3 Organic acids

Organic acids are compounds that contain carbon and are able to donate protons. These compounds are ubiquitous in living, carbon-based systems. Familiar examples, such as pyruvic acid, citric acid, succinic acid, and oxaloacetic acid are found in the energy-producing reactions of glycolysis and the tricarboxylic acid cycle and are essential to life. Humans have employed organic acids, primarily monocarboxylic acids and their derivatives, for their beneficial effects as antimicrobials in food manufacturing to prevent deterioration and extend shelf life of food products (Cherrington et al., 1991, as cited in (Ricke, 2003)). Organic acids are also a vital part of chemical industries. For instance, lactic acid has served as a building block for biodegradable plastic production, citric acid has been employed in environmental remediation, and fumaric acid has been used to produce polyesters and other synthetic polymers (Tsao, 1999). Organic acids, including oxalic acid, citric acid, hippuric acid, glucuronic acid, and aconitic acid, are normally produced and excreted by humans (Lawson, 1976). However, some organic acids, when present in abnormal concentrations within the body, produce harmful effects.

2.3.1 Detrimental organic acids in biological fluids

Organic acids have been measured in numerous biological fluids, and their measurement is significant within the wide context of health and disease. Dental health researchers have reported organic acids in oral fluid within the context of dental caries (Linke & Moss, 1992). Evaluation of exposure to environmental pollutants and of the glutathione antioxidant system can be based on urinary organic acid profiles (Lord, 2008). In medicine, inborn errors of organic acid metabolism, many of which cause neurological disease (Marsden, 1992), are screened and diagnosed using organic acid analysis of urine (Lehotay, 1995), as well as serum and CSF (Hoffmann, 1993).

Organic acids are present in the CSF and can therefore affect brain function (Hoffmann, 1993). Neurological effects associated with organic acidosis include headaches, seizures,

intermittent esotropia, and extremity paralysis (Hoffmann, Aramaki, Blum-Hoffmann, Nyhan, & Sweetman, 1989). These organic acids are not necessarily of somatic origin or transported from the blood to the CSF, and some organic acids within the CSF are thought to originate from cerebral metabolism (Hoffmann, 1993). In particular, short-chain monocarboxylic acids are capable of crossing the blood-brain barrier by facilitated transport; however, the mechanism is saturable and, in the case of lactate, stereospecific (Oldendorf, 1973). This limits the amount of organic acids transported from blood to the CSF. On the other hand, 6-, 8-, and 10-carbon fatty acids pass more freely through membranes and cross the blood-brain barrier by diffusion as a result of lipid-solubility (Oldendorf, 1973).

Lactic acidosis is one of the most common and severe forms of acidosis (Gauthier, 2002), and up to 3.8% of hospitalized patients suffer from lactic acidosis (Luft, 1983). The significance of this is underscored by the reported 30 – 88% mortality rate associated with lactic acidosis in hospitalized patients (Luft, 1983). In the agriculture sector, lactic acidosis is highly important for the cattle industry. Diarrhea is a major cause of death in neonatal calves (Ott, 1993), and lactic acidosis is a primary biochemical abnormality associated with diarrhea, as reviewed by (Grove-White, 1998; Kaae & de Morais, 2008; Kasari, 1999). While lactic acid appears to be primarily important, acetic acid and pyruvic acid also contribute to diarrheic acidosis in calves (Omole et al., 2001) and presumably in human infants (Ewaschuk, 2004). There may also be other organic acids that contribute to the acidosis. The prevalent and detrimental nature of metabolic acidosis in both animals and humans indicates the need to characterize this disorder as thoroughly as possible.

2.4 Measurement of organic acids in biological fluids

2.4.1 High performance liquid chromatography

HPLC is a form of column chromatography used to identify, quantify, and purify molecules of interest (Bird, 1989). With this technique, a complex liquid mixture of molecules, such as a biological fluid, is injected into a continuous stream of liquid mobile phase and carried through a hollow, resin-filled column. Inside the column, individual molecules are separated, and subsequently analyzed, from one another based on the molecules' dissimilar interactions with the liquid mobile phase and the stationary phase (resin) (Bird, 1989). Molecules can be separated

based on their solubility in water (normal phase chromatography), solubility in organic solvents (reverse phase chromatography), ionic charge, and size (Bird, 1989).

HPLC column resin must optimally increase the interaction between the solute of interest and the resin particles to achieve separation of the solute from other molecules in a complex mixture (Bird, 1989). The use of small resin particles increases the resin surface area with which the solute can interact and reduces the solvent volume between resin particles, thereby facilitating the interaction (Bird, 1989). Column resins must be able to withstand the high pressures (1700 – 20,000 kPa) created as a consequence of reduced resin particle size, and silica is most commonly used for this purpose (Lough & Wainer, 1996). Silica is used either unchanged or altered via chemical modification of the silanol groups, depending on the solutes of interest (Lough & Wainer, 1996). The silanol groups within a modified silica-based column can never be completely chemically modified, with up to 30% remaining as free silanol, and this can lead to irreproducible HPLC results (Lough & Wainer, 1996). Non-silica-based HPLC column resins, including styrene-divinylbenzene copolymer, overcome some of the disadvantages of silica-based resins and also provide stability over a wider pH range than silica-based resins (Lough & Wainer, 1996).

2.4.1.1 High performance liquid chromatography of organic acids

HPLC has been used to measure organic acids in biological fluids of calves. In recent years, several authors (Abeysekara, 2009; Ewaschuk, 2004; Omole, 1999) have studied lactate enantiomers and other organic acids, such as acetate and pyruvate, with HPLC assays of calf biological fluids. Omole et al. (Omole, Brocks, Nappert, Naylor, & Zello, 1999) developed an HPLC method for measuring lactic acid enantiomers in calf serum to help determine the origin of lactic acidosis in diarrheic calves. The utility of HPLC was expanded by Ewaschuk et al. (Ewaschuk, Naylor, Barabash, & Zello, 2004), who validated HPLC organic acid measurement in calf rumen fluid, feces, and urine. Ewaschuk et al. (Ewaschuk, Naylor, Palmer et al., 2004) applied this method to measure organic acids in rumen fluid, feces, serum, and urine from calves and discovered that D-lactic acid was over-produced in the colon of diarrheic calves. Abeysekara et al. (Abeysekara et al., 2007) extended the method further by using HPLC to measure organic acids in cerebrospinal fluid of acid-infused calves to determine that infused D-lactic acid

impaired central nervous system function despite creating a lesser acidemia than other infused acids.

2.4.1.2 Biological sample preparation for high performance liquid chromatography analysis of organic acids

Biological fluid samples require preparatory treatment prior to chromatographic analysis for organic acids. Preparatory steps are required to remove interfering substances, concentrate analytes, arrest enzymatic processes, and reduce contamination of chromatographic columns (Ewaschuk et al., 2002). Several methods exist for the preparation of biological fluids prior to chromatographic analysis. These include distillation and extraction, dialysis, centrifugation, and ultrafiltration (Ewaschuk et al., 2002).

Ultrafiltration through a filter with a molecular weight cut off (MWCO) of 5,000, using centrifugation at 5,500 x *g*, is a simple, rapid, accurate, and reliable method that has been used to prepare biological fluid samples prior to HPLC. Omole et al. (Omole et al., 1999) prepared calf serum for HPLC analysis of lactic acid using ultrafiltration and reported the technique to be fast and simple with excellent recovery of the organic acid being investigated. Ewaschuk et al. (Ewaschuk, Naylor, Barabash et al., 2004) validated the use of this method for preparation of calf rumen fluid, feces, and urine for HPLC analysis of organic acids with good results. Recently, Abeysekara et al. (Abeysekara et al., 2007) employed this method to prepare calf cerebrospinal fluid prior to HPLC analysis of D- and L-lactic acid.

2.4.2 Mass spectrometry of organic acids

Gas chromatography and mass spectrometry (GC/MS) is widely used for urinalysis in clinical medicine to aid the diagnosis of diseases characterized by metabolic derangements, including elevated urinary organic acids (Chace, 2003). GC/MS sample preparatory steps include solvent extraction of organic acids from urine and derivitization using trimethylsilyl or tert-butyldimethylsilyl, which is necessary to increase the volatility of intended analytes (Chace, 2003). Tandem mass spectrometry (MS/MS) has been described as one of the most important advancements in neonatal screening for metabolic disorders in the past half-century (Chace, 2003). It has the advantages over GC/MS of not requiring derivitization prior to analysis, having the ability to measure multiple analytes from different chemical families (and thus screening for

multiple metabolic disorders) in a single analytical run, and facilitating higher throughput, all of which have proved important for screening large populations for metabolic disorders (Chace, 2003). Although the initial equipment costs are higher for MS/MS compared to GC/MS, the running costs are lower (Kawana, 2008).

MS/MS can be used to help identify biological macromolecules, via comparison of mass spectra from an unknown to those of unknown compounds, or by deduction using known molecular masses of fragmentable groups as seen on mass spectra of unknown compounds. For example, two fragments on a mass spectrum that differ in m/z by 18 Da or 44 Da can indicate loss of a water molecule or a CO_2 molecule (Reinnig, 2008). There are several computer programs to assist identification of molecules, as well. For example, one program (Colby College Chemistry, 1998) generates putative molecular formulae based on input information of parent peak mass and fragment masses, while another program (Colby College Chemistry, 1997) generates possible molecular formulae of fragment ions based on input fragment molecular masses.

3. MATERIALS AND METHODS

3.1 Sample collection

Biological fluids were obtained from previous investigations (Abeysekara, 2009; Ewaschuk, 2004; Omole, 1999) and stored at -20°C until the present analysis. Details describing the origin of the samples are presented in Figure 3.1.

Omole's (Omole, 1999) research provided serum from diarrheic and healthy calves, ranging in age from 1 to 45 days, which were selected from the University of Saskatchewan's WCVM veterinary clinic and Goodale Research Farm, respectively. The mean serum anion gap was 19.7 mM in the diarrheic group and 5.6mM in the healthy group (Omole, 1999). The mean serum D-lactate concentration was 5.1 mM in the diarrheic calves and not quantifiable in the healthy calves (Omole, 1999). Omole (Omole, 1999) also reported an unidentified peak, which was higher in the diarrheic group and correlated with the anion gap, on his HPLC chromatograms. Ninety percent of the diarrheic calves presented to the clinic in a depressed state (Omole, 1999). Venous samples were obtained during the spring of 1997 from the diarrheic (n = 12) and healthy (n = 13) calves. Blood was drawn from the jugular vein and allowed to clot at room temperature for 20 to 40 minutes. Serum was separated by centrifugation and frozen at -20°C until assayed.

Ewaschuk's (Ewaschuk, 2004) research provided serum, urine, feces, and rumen fluid from healthy and diarrheic calves, less than 35 days old, that were selected from the University of Saskatchewan's Goodale Research Farm and WCVM veterinary clinic, respectively (Ewaschuk, 2004). The mean serum anion gap was 28.4 mM in Ewaschuk's diarrheic calves and 5.6 mM in the healthy calves. Diarrheic calves had significantly higher concentration of D-lactate in serum, urine, feces, and rumen fluid (Ewaschuk, 2004). Blood was drawn from the jugular vein of the healthy (n = 11) and diarrheic (n = 69) calves, allowed to clot for 20 minutes at room temperature, and centrifuged. Serum was drawn off and stored at -20°C until assayed. Feces was collected from the healthy (n = 11) and diarrheic (n = 14) calves following perineal massage, mixed with thiomersal (250 µmol/L) as a bacteriostatic agent, and frozen at -20°C until assayed. Rumen fluid was suctioned via the mouth from the healthy (n = 10) and diarrheic (n = 13) calves with the tip and tubing of a human enema kit. Rumen fluid was mixed with thiomersal (250 µmol/L) as a bacteriostatic agent, and frozen at -20°C until assayed. Urine was collected at admission from healthy (n = 10) and diarrheic (n = 14) calves and frozen at -20°C until assayed.

Abeysekara's (Abeysekara, 2009) research provided serum and cerebrospinal fluid (CSF) from acid-infused calves and saline-infused calves. The calves were healthy male Holstein calves housed in the Dairy Barn, Department of Animal and Poultry Sciences, at the University of Saskatchewan (Abeysekara, 2009). Serum was obtained from HCl-infused calves (n = 16), D-LA-infused calves (n = 12), L-LA-infused calves (n = 5), and saline-infused calves (n = 6). CSF was obtained from HCl-infused calves (n = 16), D-LA-infused calves (n = 12), L-LA-infused calves (n = 5), and saline-infused calves (n = 6).

Ewaschuk's (Ewaschuk, 2004) research also provided serum from diarrheic piglets (n = 30) ranging from one to four days old, diarrheic foals (n = 18) aged less than 30 days, and diarrheic human infants (n = 22) less than 3 years old. D-lactate was quantifiable in one foal, not detected in any of the piglets, and detected but not quantifiable in one human infant (Ewaschuk, 2004). Diarrheic piglets were housed at the Prairie Swine Centre (Saskatoon, Saskatchewan) and were chosen based on the presence of diarrhea severe enough to warrant treatment. The diarrheic foals were selected from the University of Saskatchewan's WCVM large animal teaching hospital or from Haygard, Dvidson, McGee and Associates of Lexington, KY. Diarrheic human infants were selected from the Royal University Hospital (Saskatoon, SK) based on the presence of diarrhea severe enough to warrant serum electrolyte measurements (Ewaschuk, 2004).

3.2 Experimental design

Compound X was measured in biological fluids using HPLC (section 3.4). The details of the analyses, each pertaining to a specific objective (section 1.2), follow in sections 3.2.1 – 3.2.5. The experimental design is outlined in Figure 3.1.

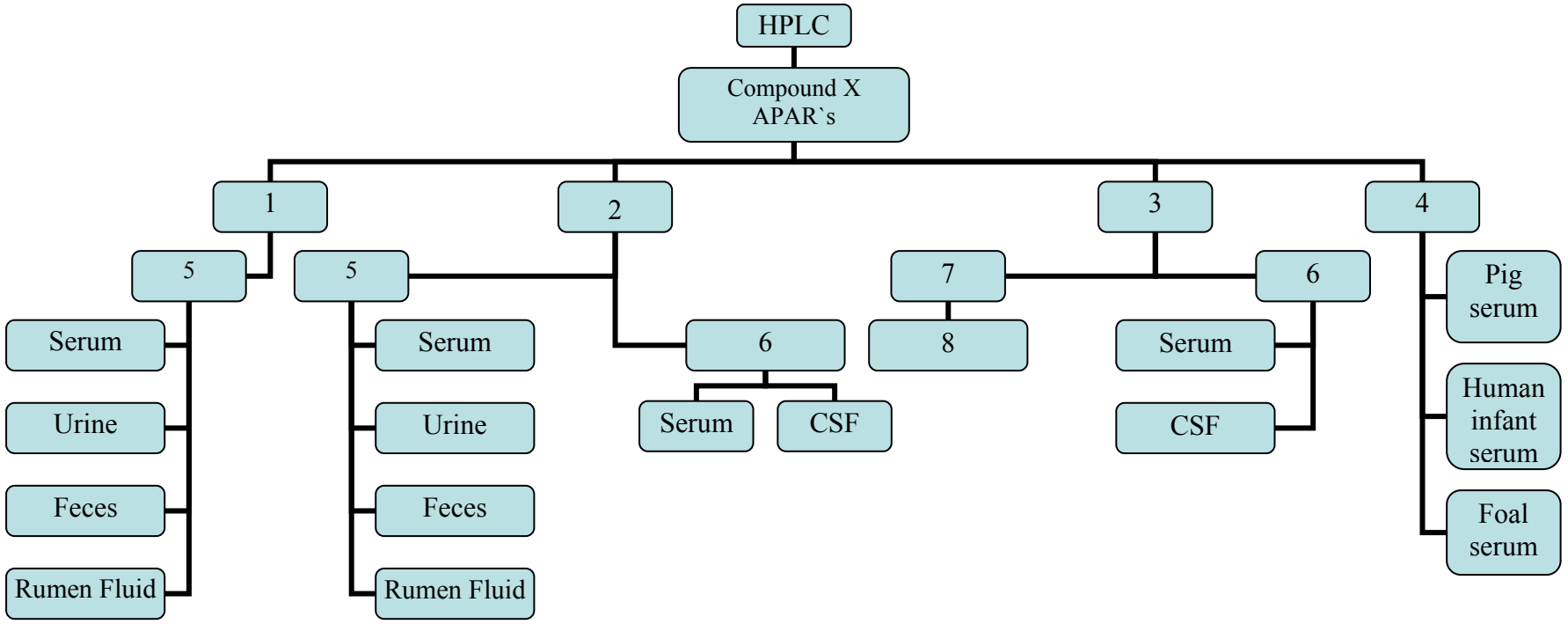


Figure 3.1. Experimental design used to characterize Compound X.
1, Compound X related to Diarrhea
2, Compound X vs. D-lactate
3, Compound X vs. neurological disturbance
4, Compound X in other species
5, Healthy vs. diarrheic calves
6, Healthy calves infused with saline, hydrochloric acid, L-lactic acid, or DL-lactic acid
7, Diarrheic calves
8, Able to stand at presentation vs. unable to stand at presentation

3.2.1 Compound X related to diarrheal illness

The relationship between Compound X and diarrheal illness was determined by comparing Compound X average peak area ratios (APAR's) from diarrheic with those of healthy calves. Comparisons were made in serum, urine, feces, and rumen fluid.

3.2.2 Compound X related to D-lactic acidosis

The association between Compound X and D-lactic acidosis was determined by comparing Compound X APAR's with D-lactic acid concentrations from diarrheic and healthy calves. Serum, urine, feces, and rumen fluid were analyzed.

The correlation between Compound X and D-lactic acidosis was further evaluated by comparing Compound X APAR's from calves infused with saline, hydrochloric acid, L-lactic acid, and DL-lactic acid.

3.2.3 Compound X related to acidosis-associated neurological disturbance

Amongst diarrheic calves, the relationship between Compound X and neurological disturbance was evaluated by comparing the Compound X APAR's of two categories of diarrheic calves – those who presented to the clinic standing (normal neurological status) and those who presented in lateral or sternal recumbency (abnormal neurological status). Information about the calves' presentation status was obtained from their medical records.

Abeysekara's (Abeysekara, 2009) calves that were infused with saline, hydrochloric acid, L-lactic acid, or DL-lactic acid were assigned a numerical neurological score. In these calves, the relationship between Compound X and neurological disturbance was evaluated by comparing the Compound X APAR's with the neurological scores that were determined during the experimental infusions.

3.2.4 Compound X in other species

The presence of Compound X in diarrheic foals, piglets, and human infants, was determined by measuring the APAR for these samples. Compound X levels were compared between these groups and calves to determine relative levels.

3.2.5 Attempts to identify compound X

Techniques employed to attempt to identify Compound X included gas chromatography with mass spectrometry (GC/MS), liquid chromatography with tandem mass spectrometry (LC/MS/MS), and Fourier-transform mass spectrometry (FTMS).

3.3 Materials and Chemicals

HPLC grade phosphoric acid (85%) was obtained from EMD Chemicals (Gibbstown, New Jersey, USA). Sterile filtered bovine calf serum was obtained from HyClone (Logan, Utah, USA). Adipic acid (internal standard) was obtained from Sigma (St. Louis, MO, USA). Modified polyethersulfone microcentrifugal filtration units (10 K MWCO) were obtained from VWR International (Edmonton, AB). Thirteen millimeter polysulfone syringe filters (0.2 μm) were obtained from Whatman (Maidstone, Kent, UK) and 10 mL Luer-Lok™ disposable syringes from the National Scientific Company (Rockwood, TN, USA).

3.4 Chromatographic analysis

Compound X, whose identity was unknown, was measured semi-quantitatively using high performance liquid chromatography. Since the identity of Compound X was unknown, the actual concentration in each sample could not be determined. Instead, an internal standard (7 mM adipic acid) was added to each sample prior to HPLC analysis, and the peak area ratio (PAR) of Compound X to adipic acid was calculated. All injections were done in duplicate, which allowed calculation of the average peak area ratio (APAR) for each set of duplicate injections. The APAR was used as a semi-quantitative measure of Compound X.

The Waters HPLC system included a Waters 600 pump, a 486 UV absorbance detector, and a 710 Ultra WISP autoinjector (Waters, Mississauga, ON). Data collection and integration were performed using Waters Millennium®32 chromatography manager (Waters, Mississauga, ON).

Chromatographic separation of Compound X and adipic acid (internal standard) was achieved with a Shodex RSpak KC-G guard column combined with an ion-exclusion, reversed-phase 300 x 8.0 mm analytical column (Shodex RSpak KC-8111, Showa Denko K.K., Kawasaki, Japan). Mobile phase, consisting of 0.1% phosphoric acid (0.1% formic acid was used for HPLC fraction collection for mass spectrometric analyses) in Milli-Q purified water, was filtered

through a 0.45 μm membrane filter and degassed under vacuum to remove contaminants and oxygen. The mobile phase was pumped at 0.7 mL/min for 28 minutes per injection. The column temperature was held constant at 50°C and UV detection was at 205 nm. The sample injection volume was 20 μL for all injections, and all injections were done in duplicate (Omole, 1999).

The presence of Compound X was designated by a signal to noise ratio greater than or equal to 5:1 on the chromatogram.

3.5 HPLC sample preparation

Samples were prepared by combining 100 μL of the biological fluid with 50 μL of 7 mmol/L adipic acid (internal standard) and 50 μL of Milli-Q water. Samples were filtered through VWR microcentrifugal filtration units at 14,000 x g for 30 minutes and the filtrate was injected into the HPLC system (Ewaschuk, 2004).

3.5.1 Serum samples

No pre-preparatory steps were required for serum before microcentrifugal filtration.

3.5.2 Urine samples

Urine was thawed at room temperature and diluted 1 in 5 with 0.1% phosphoric acid prior to microcentrifugal filtration (Ewaschuk, 2004).

3.5.3 Fecal samples

Feces was thawed at 4°C and shaken for 20 minutes on an automatic shaker. One gram of feces was combined with 9 mL of Milli-Q water, homogenized for one minute, and centrifuged at 17,000 x g for 30 minutes. The supernatant was passed through a polysulfone syringe filter (0.2 μm) prior to combining with 50 μL of internal standard and 50 μL of Milli-Q water (J. B. Ewaschuk, 2004).

3.5.4 Rumen fluid samples

Rumen fluid was thawed at 4°C and shaken for 20 minutes on an automatic shaker. One gram of rumen fluid was combined with 9 mL of Milli-Q water, homogenized for one minute, and centrifuged at 17,000 x g for 30 minutes. The supernatant was passed through a polysulfone

syringe filter (0.2 μm) prior to combining with 50 μL of internal standard and 50 μL of Milli-Q water (Ewaschuk, 2004).

3.5.5 Cerebrospinal fluid samples

No pre-preparatory steps were required for cerebrospinal fluid prior to microcentrifugal filtration.

3.6 Mass spectrometric analysis

3.6.1 Gas chromatography with mass spectrometry

Gas chromatography with mass spectrometry (GC/MS) was performed with a HP 7890 gas chromatograph interfaced with a HP 5975C mass detector operating in total ion current mode. The gas chromatograph was equipped with a HP-5 column with specifications of 0.25 mm ID, 0.25 μm film thickness, and 30 m length. The system was controlled with HP MSD ChemStation software.

A gas chromatogram was obtained for a pooled HPLC urine fraction containing a large amount of Compound X. A mass spectrum was generated for each peak on the gas chromatogram. Peak identification was accomplished by comparing with three silylated organic acid libraries.

3.6.2 Liquid chromatography with tandem mass spectrometry

Liquid chromatography with tandem mass spectrometry (LC/MS/MS) was performed using a QSTAR® XL Hybrid LC/MS/MS System, which employs a quadrupole time-of-flight mass spectrometer. The specified resolution of this instrument is 8,000 (Full-width half-height maximum, FWHM) (Applied Biosystems).

Mass spectra were obtained for a pooled HPLC urine fraction collection containing a large amount of Compound X (high sample) and a pooled HPLC urine fraction collection containing a small amount of Compound X (low sample). The mass spectra from the high and low samples were compared to identify m/z peaks that fit the pattern of high intensity in the "high sample" and low intensity in the "low" sample. The molecular weights corresponding to m/z peaks that fit this pattern were searched in the Dictionary of Natural Products (Taylor & Francis Group, 2010), a subset of the Chapman & Hall/CRC Chemical Database. The output

results were examined for potentially relevant compounds within the context of diarrheal illness and metabolic acidosis.

Compound X was predicted to be a physiologically-relevant small organic acid or organic acid derivative, as it was first observed by Omole (Omole, 1999) in calf serum using an organic acid-separating HPLC column with UV detection at 205 nm, which he determined to be the absorption maxima for lactic acid. Additionally, Compound X absorbs light at 205 nm, and all compounds with double bonds, including but not limited to carboxylic acids, absorb light in the ultraviolet range between 200 and 350 nm (Scott, 2003).

3.6.3 Fourier-transform mass spectrometry

FTMS was performed using a Bruker Daltonics 7T Apex III mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The ion source was a Bruker Apollo ESI.

The Compound X peak fractions from 20 injections from a urine sample containing a high level of Compound X were collected and pooled using HPLC. The sample was analyzed using Fourier-transform mass spectrometry (FTMS) in positive ESI mode.

The accurate masses corresponding to the FTMS m/z ratios were searched in the Dictionary of Natural Products (Taylor & Francis Group, 2010), a subset of the Chapman & Hall/CRC Chemical Database, using a 10 ppm range. When matches were obtained in this range, the search was repeated using a 5 ppm and then a 2 ppm range to narrow the results. Compounds that matched at this narrow range were deemed as potential candidates for Compound X.

3.7 Mass spectrometry sample preparation

3.7.1 Gas chromatography with mass spectrometry

The Compound X peak fractions from 20 injections from a urine sample containing a high level of Compound X were collected and pooled using HPLC. This was accomplished by manually collecting, in a glass vial, the portion of the HPLC effluent that contained Compound X from 20 successive injections. The timing (in seconds after noticing a rise in absorbance on the UV detector) of the collection was based on the HPLC mobile phase flow rate (mL/sec) and the volume (mL) of the effluent line extending from the UV detector. This allowed Compound X to be collected in isolation from other components in the urine.

Solvent extraction and was performed on the pooled fraction. The pooled fraction was combined with tropic acid (200 µg/mL) as the internal standard. Methoxyamine (2%) was added and the pH was reduced to less than 1 with HCl. An excess of sodium chloride was added. Ethyl acetate was combined with the mixture, and it was shaken and centrifuged for three minutes. The organic layer was removed and added to anhydrous sodium sulfate and shaken. The resulting organic layer was removed and combined with diethyl ether. The final organic layer was transferred to a test tube and evaporated to dryness with gentle heat under nitrogen.

Derivatization was accomplished by adding bistrimethylsilylfluoroacetamide with 1% trimethylchlorosilane to the dry tubes containing extracted organic acids and heating at 70°C for 30 minutes. One microlitre was injected into the GC/MS system.

3.7.2 Liquid chromatography with tandem mass spectrometry

The Compound X peak fractions from 20 injections from a urine sample containing a high level of Compound X were collected and pooled (high sample) using HPLC. This was accomplished by manually collecting, in a glass vial, the portion of the HPLC effluent that contained Compound X from 20 successive injections. The timing (in seconds after noticing a rise in absorbance on the UV detector) of the collection was based on the HPLC mobile phase flow rate (mL/sec) and the volume (mL) of the effluent line extending from the UV detector. This allowed Compound X to be collected in isolation from other components in the urine. Similarly, the Compound X peak fractions from 20 injections from a urine sample containing a low level of Compound X were collected and pooled (low sample) using HPLC. The high sample and low sample were analyzed using LC/MS/MS in negative electrospray ionization (ESI) mode.

3.7.3 Fourier-transform mass spectrometry

The Compound X peak fractions from 20 injections from a urine sample containing a high level of Compound X were collected and pooled using HPLC. The process to obtain this sample was identical to that described in section 3.7.1. The pooled sample was analyzed using Fourier-transform mass spectrometry (FTMS) in positive ESI mode.

3.8 Statistical analysis

Statistical analyses were performed using SPSS v.16.0 for Microsoft Windows (Chicago, IL). Statistical significance was designated as $p < 0.05$.

3.8.1 Compound X related to diarrheal illness

Mann-Whitney U tests were used to determine statistical differences between Compound X APAR's from healthy and diarrheic calf serum, urine, feces, and rumen fluid. A non-parametric test was used because the Q-Q plots for these variables showed that the data were not normally distributed.

3.8.2 Compound X related to D-lactic acidosis

Mann-Whitney U tests were used to determine statistical differences between Compound X APAR's amongst the four groups of infused calves (saline, hydrochloric acid, L-lactic acid, and DL-lactic acid) in serum and CSF. Spearman's rho was used to determine correlations between Compound X and D-lactate in diarrheic and healthy calf serum, urine, feces, and rumen fluid. Spearman's rho was used to determine correlations between Compound X and D-lactate in serum and cerebrospinal fluid from the infused calves (all four groups of infused calves were combined for this analysis). The non-parametric Mann-Whitney U test was chosen due to small sample sizes ($5 \leq n \leq 15$ for infused calves) and because Q-Q plots showed that the Compound X APAR data did not fit the normal distribution.

3.8.3 Compound X related to acidosis-associated neurological disturbance

A Mann-Whitney U test was used to detect a statistical difference between diarrheic calves with normal neurological presentation and those with abnormal neurological presentation. The non-parametric test was used because of the small sample size ($n = 12$) of the group that was neurologically normal and because the Q-Q plots showed the Compound X APAR data did not fit the normal distribution.

Spearman's rho correlation was used to determine correlations in infused calves (all four groups of calves were combined for this analysis) between serum Compound X and neurological score, as well as between cerebrospinal fluid Compound X and neurological score. This non-

parametric correlation test was used due to small sample sizes ($4 \leq n \leq 14$) and data that did not fit the normal distribution, as determined by Q-Q plots.

3.8.4 Compound X in other species

Mann-Whitney U tests were performed to determine statistical differences between Compound X APAR's amongst diarrheic human infants, diarrheic calves, diarrheic foals, and diarrheic piglets. A non-parametric statistical test was employed because the data were not normally distributed, as tested by Q-Q plots.

4. RESULTS

Compound X was present in 411 of 414 (99.3%) samples analyzed. Compound X was absent in one healthy calf rumen sample (n = 11) and 3 diarrheic calf rumen samples (n = 23).

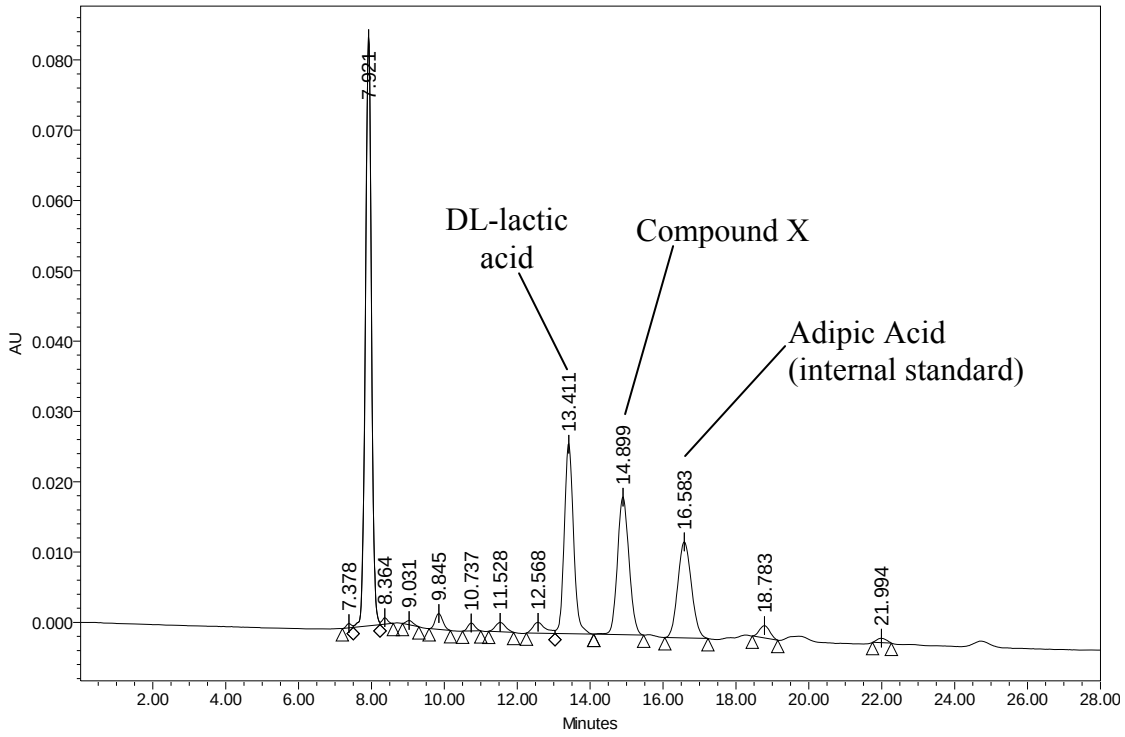


Figure 4.1. A typical high performance liquid chromatogram of standard calf serum with adipic acid added as an internal standard. Numbers above peaks represent retention times in minutes for chemical components of the serum.

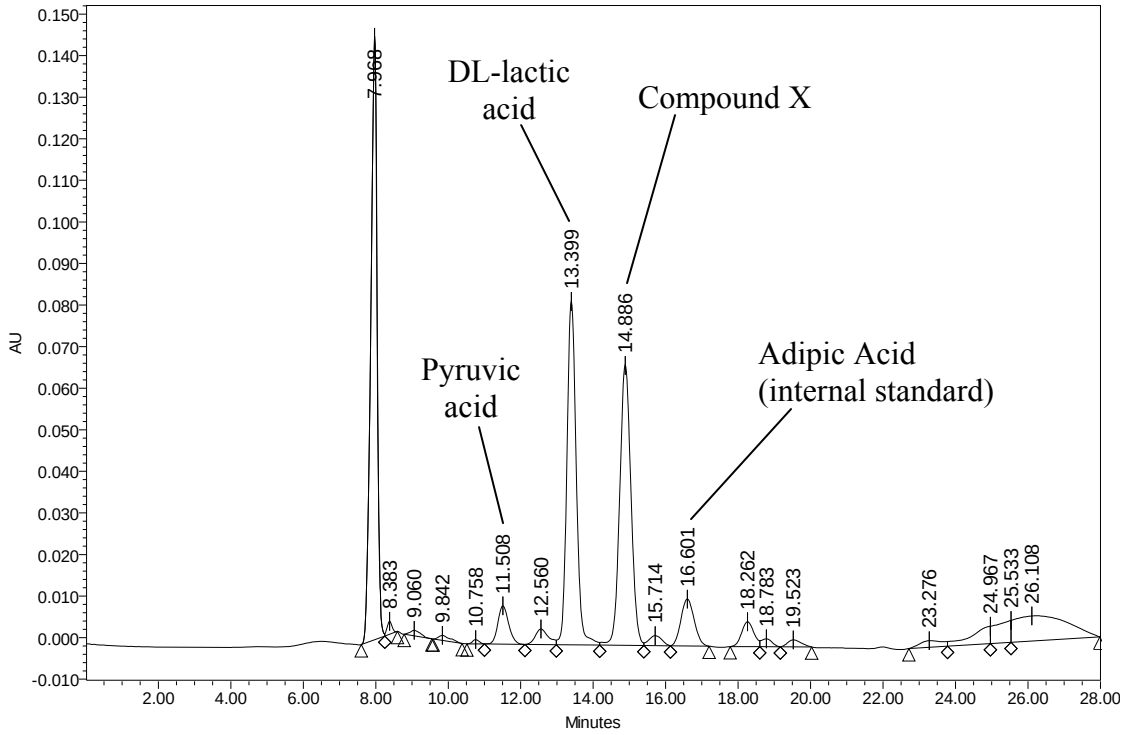


Figure 4.2. A typical high performance liquid chromatogram of a diarrheic calf serum sample with adipic acid added as an internal standard. Numbers above peaks represent retention times in minutes for chemical components of the serum.

4.1 Compound X related to diarrheal illness

In healthy calves, the Compound X concentration was greatest in feces, urine, and rumen fluid (Figure 4.3). There were no statistically significant differences between these three biological matrices in healthy calves. The concentration was statistically significantly ($p<0.05$) lower in serum than feces, urine, and rumen fluid.

In diarrheic calves, the Compound X concentration was greatest in urine, followed by feces, serum, and rumen fluid (Figure 4.3). The difference between each biological fluid was statistically significant ($p<0.05$).

Both feces and rumen fluid from healthy calves contained a higher concentration of Compound X than feces ($p<0.05$) and rumen fluid ($p<0.05$) from diarrheic calves, while healthy calf serum had a lower Compound X concentration than diarrheic calf serum ($p<0.05$) (Figure 4.3). There was no statistically significant difference between healthy calf urine and diarrheic calf urine.

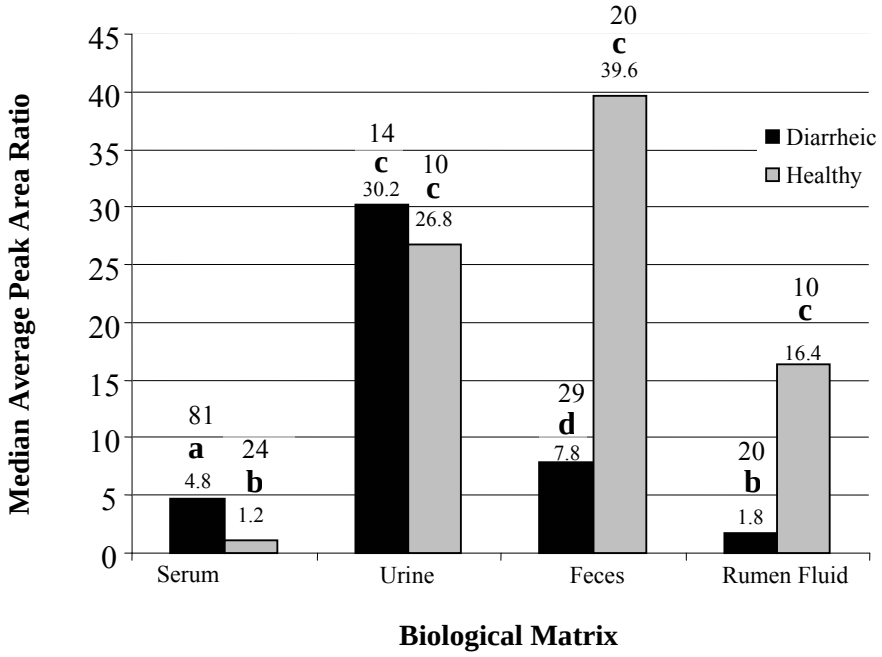


Figure 4.3. Comparison of the median average peak area ratios (numbers directly above bars) from biological matrices of healthy and diarrheic calves. Letters above bars indicate statistical differences between bars, as determined by Mann-Whitney U tests (any bars with the same letter are not different, while any two bars with different letters are significantly different, $p<0.05$). Whole numbers above bars indicate sample size (n).

Table 4.1. Comparison of Compound X mean average peak area ratios (APAR) in healthy and diarrheic calf biological fluids.

Biological Fluid	Healthy (mean ± SD, n)	Diarrheic (mean ± SD, n)	p-value[a]
Serum	1.32 ± 0.60, 24	6.35 ± 4.80, 80	<0.05
Urine	44.9 ± 60.3, 10	41.6 ± 46.5, 14	1
Feces	42.7 ± 30.7, 20	11.1 ± 10.1, 29	<0.05
Rumen Fluid	26.1 ± 26.2, 10	4.48 ± 8.60, 20	<0.05

[a] p-values were determined using the non-parametric Mann-Whitney U test.

4.2 Compound X related to D-lactic acidosis

Healthy calves that were experimentally infused with saline, hydrochloric acid (HCl), DL-lactic acid (DL-LA), or L-lactic acid (L-LA) had relatively low levels of Compound X in serum and cerebrospinal fluid (CSF). The serum Compound X APAR's from these calves ranged from 1.45 to 1.87. These APAR's were slightly higher than the median APAR (1.2) in healthy calves, but lower than the median APAR (4.8) in diarrheic calves (Figures 4.3 and 4.4).

HCl- and DL-LA-infused calves had higher serum levels of Compound X than those infused with saline and L-LA. There was no statistically significant difference between the HCl- and DL-LA-infused calves, nor was there a statistically significant difference between the saline- and L-LA-infused calves (Figure 4.4).

Healthy calves that were experimentally infused with saline, HCl, L-LA, or DL-LA showed little variation with regard to the amount of Compound X in CSF. Compound X was statistically significantly higher in the CSF of HCl- and DL-LA-infused calves when compared to L-LA-infused calves (Figure 4.4). However, the concentration of Compound X in CSF of HCl- and DL-LA-infused calves was not statistically significantly greater than that of saline-infused calves (Figure 4.4).

The Compound X concentration was higher in serum than CSF from experimentally infused calves for each infusate tested. Comparing serum and CSF across all infusate conditions, the Compound X concentration from DL-LA-infused calf CSF was not different from serum from saline- and L-LA-infused calves (Figure 4.4).

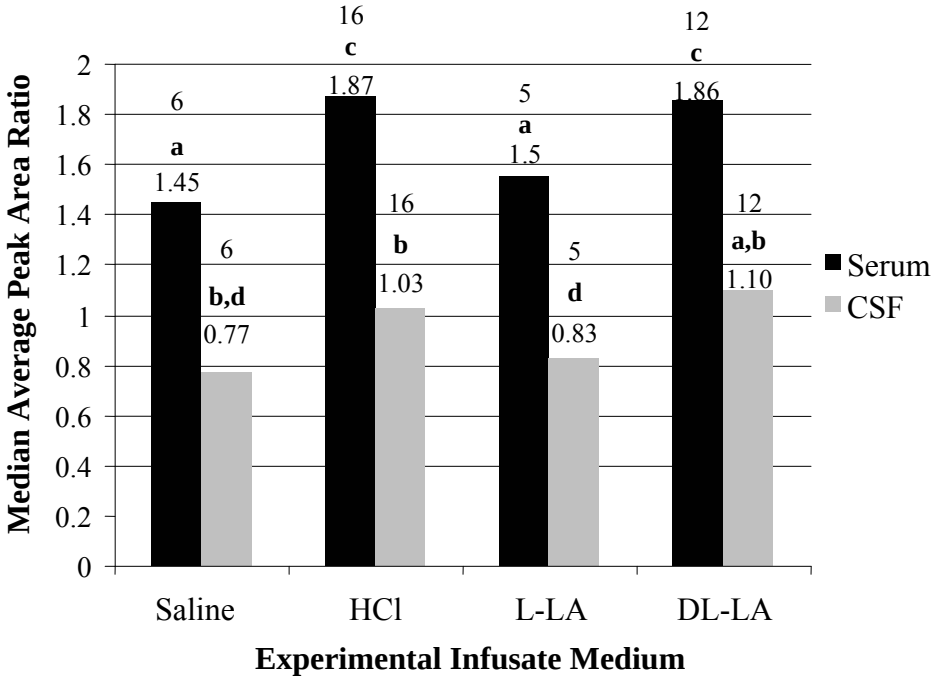


Figure 4.4. Comparison of the median average peak area ratios from serum and cerebrospinal fluid (CSF) of healthy and diarrheic calves. Numbers directly above bars are the median APARs. Letters above bars indicate statistical differences between bars, as determined by Mann-Whitney U tests (any bars with the same letter are not different, while any two bars with different letters are significantly different, $p<0.05$). Whole numbers above bars indicate sample size. HCl, hydrochloric acid; L-LA, L-lactic acid; DL-LA, DL-lactic acid.

Compound X did not correlate with D-lactate in serum or cerebrospinal fluid from infused (all four infusion conditions combined) calves (Table 4.2).

In diarrheic and healthy calf serum, Compound X correlated with D-lactate concentration (Spearman's rho = 0.289, p = 0.003) when the two groups of calves were considered together (Table 4.2). No significant correlations were observed between Compound X and D-lactate in diarrheic or healthy calf serum when these were considered as separate groups (Table 4.2). In rumen fluid, Compound X negatively correlated with D-lactate (Spearman's rho = -0.389, p = 0.03) when diarrheic and healthy calves were combined (Table 4.2). There were no significant correlations between Compound X and D-lactate in diarrheic or healthy calf rumen fluid when these were considered as separate groups (Table 4.2). No statistically significant correlations were found between Compound X and D-lactate in urine or feces from diarrheic and healthy calves (Table 4.2).

Table 4.2. Correlations between Compound X and D-lactate in calf biological fluids.

Biological Fluid Analyzed	n	Spearman's rho	p-value
Diarrheic calf serum	69	ns	0.1
Healthy calf serum	24	ns	0.06
All diarrheic & healthy calf serum	**93**	**0.289**	**0.003**
Diarrheic calf urine	14	ns	0.38
Healthy calf urine	10	ns	0.36
All diarrheic & healthy calf urine	24	ns	0.45
Diarrheic calf feces	14	ns	0.23
Healthy calf feces	11	ns	0.14
All diarrheic & healthy calf feces	25	ns	0.09
Diarrheic calf rumen fluid	13	ns	0.16
Healthy calf rumen fluid	10	ns	0.07
All diarrheic & healthy calf rumen fluid	**23**	**-0.389**	**0.03**
Infused calf serum	34	ns	0.14
Infused calf cerebrospinal fluid	29	ns	0.2

4.3 Compound X related to acidosis-associated neurological disturbance

Amongst 51 diarrheic calves, 39 presented in lateral of sternal recumbency and were defined in the current study as having abnormal neurological status. The other 12 calves presented in a standing position and were defined as having normal neurological status. The Compound X levels between these two groups were not statistically significantly different ($p = 0.982$).

Amongst calves infused with saline, hydrochloric acid, L-lactic acid, or DL-lactic acid (all four infusion conditions combined), Compound X correlated significantly with the neurological score in both serum (Spearman's rho = 0.399, $p = 0.01$) and CSF (Spearman's rho = 0.324, $p = 0.043$).

4.4 Compound X in other species

Compound X was present in diarrheic piglets, foals, and human infants. Compound X was significantly greater in serum from diarrheic human infants than serum from diarrheic calves

(p = 0.04), diarrheic foals (p < 0.01), and diarrheic piglets (p < 0.01) (Figure 4.5). Diarrheic piglets had lower serum Compound X levels than all other diarrheic species (p < 0.01 for each comparison), as well as healthy calves (p < 0.01) (Figure 4.5).

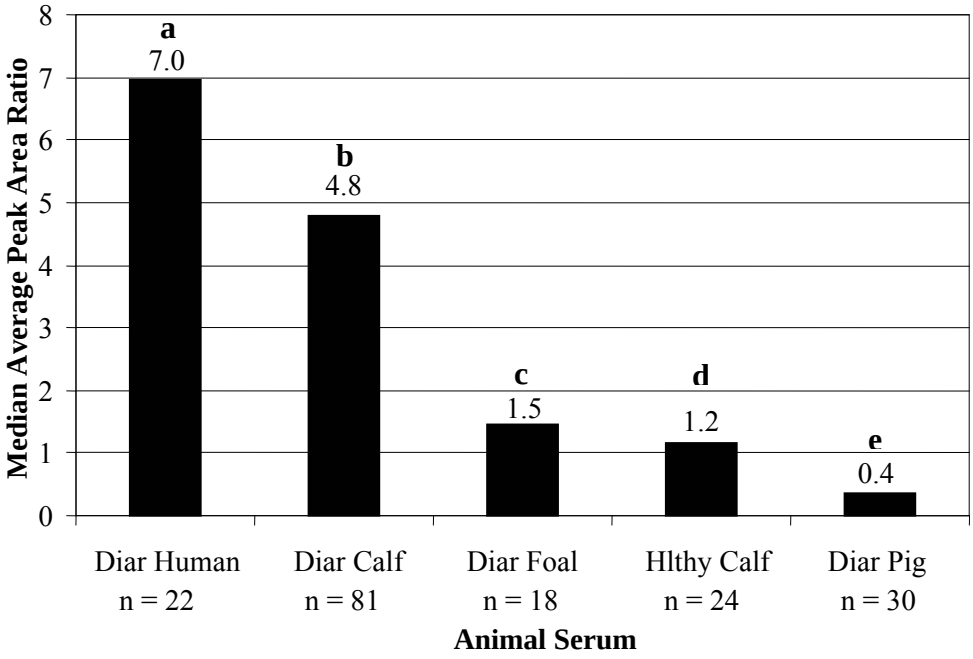


Figure 4.5. Comparison of the Compound X median average peak area ratios from serum of various species. Numbers above bars indicate median APAR. Letters above bars indicate statistical differences between bars, as determined by Mann-Whitney U tests (any bars with the same letter are not different, while any two bars with different letters are significantly different, p<0.05). Numbers above letters indicate sample size. Diar, diarrheic; Hlthy, healthy.

4.5 Attempts to identify Compound X

4.5.1 Gas chromatography with mass spectrometry

Gas chromatography with mass spectrometry (GC/MS) of a Compound X fraction collected from diarrheic calf urine revealed a very large unknown peak, which eluted at 48.1 minutes near the end of the gas chromatogram. The mass spectrum for this peak did not match any compounds within the employed databases.

4.5.2 Liquid chromatography with tandem mass spectrometry

The LC/MS/MS mass spectra from both the high sample and the low sample showed peaks near 146 m/z, 157 m/z, and 337 m/z. The 146 m/z peak in the high sample had 3-fold the intensity of the low sample. The 157 m/z peak in the high sample had 20-fold the intensity of the low sample. The 337 m/z peak in the high sample was considerably more intense than that in the low sample (Figure 4.6).

A database search for compounds corresponding to the 146 m/z peak returned 173 matches at the 1000 parts per million (ppm) level of accuracy (Table A.1 in appendix). Among these were several amino acids and amino acid derivatives. Notably, glutamic acid was among these (Table A.1 in appendix).

A database search for compounds corresponding to the 157 m/z peak returned 112 matches at the 1000 parts per million (ppm) level of accuracy (Table A.1 in appendix). Notably, butanoic acid anhydride was among these.

A database search for compounds corresponding to the 337 m/z peak returned 149 matches at the 50 ppm level of accuracy (Table A.1 in appendix). None of the matched compounds were deemed to be likely candidates for Compound X.

The mass spectrum from the high sample showed peaks at 315 m/z, 473 m/z, and 517 m/z, but these were not present on the mass spectrum from the low sample. A search of the Chapman & Hall Natural Products database returned several matches for compounds corresponding to the peaks at 315 m/z, 473 m/z, and 517 m/z (Table A.1 in appendix), though none of these were deemed to be likely candidates for Compound X.

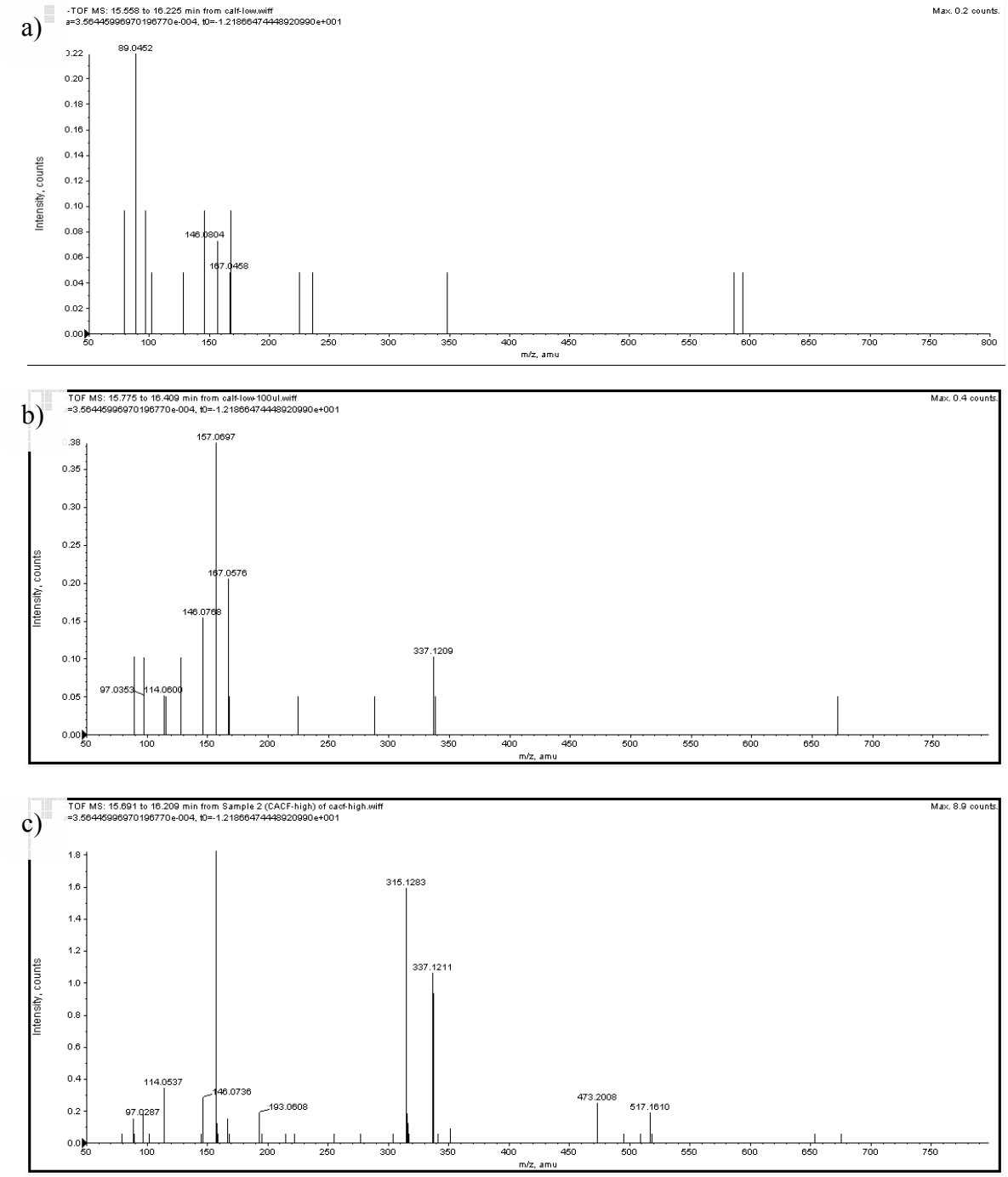


Figure 4.6. Mass spectra from injection of a)30 µL of calf urine fraction containing a small amount of Compound X; b)30 µL of a calf urine fraction containing a large amount of Compound X; c)100 µL of a calf urine fraction containing a small amount of Compound X.

4.5.3 Fourier-transform mass spectrometry

FTMS analysis identified singly-charged molecular ions at m/z 257, 317, 475, 513, 528, and 565. A search of the Chapman & Hall natural products database, using a 10 ppm window, yielded a small number of compound matches (Table 4.5). The database search was completed for the same m/z ratio peaks presuming that the ions were sodium adducts (Table 4.6) and potassium adducts (Appendix Table A.3).

Notably, the mass spectrum from FTMS showed monoisotopic peaks at 317 m/z and 475 m/z. These m/z's are similar to peaks observed on the LC/MS/MS mass spectra. An FTMS peak at 513m/z approximately corresponded to a peak at 517m/z on the LC/MS/MS mass spectra.

A search of the Chapman & Hall Natural Products database returned 130 matches for the 317 m/z using a 700 ppm accuracy range (Appendix Table A.3). A search for the 475 m/z returned 213 matches at the 250 ppm accuracy range. A search for the 513 m/z returned 16 matches at the 700 ppm accuracy range (Appendix Table A.3). None of the matched compounds for the FTMS m/z's were deemed to be likely candidates for Compound X.

Table 4.3. Compounds and corresponding molecular formulae identified as putative matches for Compound X as identified by FTMS.

Peak m/z[a]	m/z - H+	Low limit 10 ppm[b] range (molar mass)	High limit 10 ppm range (molar mass)	# of library matches
257.0300723	256.0227963	256.0202361	256.0253565	2
Compound Name			**Molecular Formula**	
Naphthaleneoctol[c]			C10H8O8	
Secobatzelline A; 4-Deimino, 4-oxo			C10H9ClN2O4	
317.0957864	316.0885104	316.0853495	316.0916713	4
Compound Name			**Molecular Formula**	
Arginine, INN, USAN; (S)-form, Mono(2-mercaptoethanesulfonate)[c]			C8H20N4O5S2	
Cyclodercitine			C19H14N3S	
Orotidine; 3N-Me, Me ester			C12H16N2O8	
Uridine-5-acetic acid; Me ester			C12H16N2O8	
475.1369619	474.1296859	474.1249446	474.1344272	5
Compound Name			**Molecular Formula**	
1,5-Anhydrofructose, 9CI; D-form, Tribenzoyl[c]			C27H22O8	
1,6-Anhydromannose; ß-D-Pyranose-form, 2,3,4-Tribenzoyl[c]			C27H22O8	
15-Chloro-1,4-epoxy-5,8,9-trihydroxy-11(13)-germacren-12,6-olid-14-oic acid; (1ß,4ß,5ß,6a,8ß,9a,10ßH)-form, 8-(2-Hydroxymethyl-2-butenoyl), Me ester[d]			C21H27ClO10	
6-Deoxymannonic acid, 9CI, 8CI; L-form, 1,5-Lactone, tribenzoyl			C27H22O8	
2,4,5-Trihydroxy-2-(hydroxymethyl)pentanoic acid; (2R,4S)-form, 1,4-Lactone, tribenzoyl[c]			C27H22O8	
513.084279	512.077003	512.0718822	512.0821238	0
528.0473004	527.0400244	527.034754	527.0452948	0
565.2863106	564.2790346	564.2733918	564.2846774	4
Compound Name			**Molecular Formula**	
Aristophyll A; 3,4-Didehydro			C34H36N4O4	
Cholestane-3,21-diol, 9CI; (3a,5a)-form, Disulfate[d]			C27H48O8S2	
Cholestane-3,21-diol, 9CI; (3ß,5a)-form, Disulfate[d]			C27H48O8S2	
Pemptoporphyrin; Di-Me ester			C34H36N4O4	

[a]m/z = mass to charge ratio of molecular ion fragments identified by the mass spectrometer
[b]ppm = parts per million
[c]also matched at 5ppm, but not at 2 ppm
[d]also matched at 5 ppm and 2 ppm

Table 4.4. Compounds and corresponding molecular formulae identified as putative matches for Compound X as identified by FTMS, assuming molecular ions were sodium adducts.

Peak m/z[a]	m/z - Na+	Low limit 10 ppm[b] range (molar mass)	High limit 10 ppm range (molar mass)	# of library matches
257.0300723	234.0408423	234.0385019	234.0431827	0
317.0957864	294.1065564	294.1036153	294.1094975	3
Compound Name			**Molecular Formula**	
Distichonic acid A[c]			C10H18N2O8	
Distichonic acid A; 2'-Deoxy, 3-hydroxy[c]			C10H18N2O8	
Distichonic acid A; 2'-Epimer[c]			C10H18N2O8	
475.1369619	452.1477319	452.1432104	452.1522534	9
Compound Name			**Molecular Formula**	
Aiphanol[c]			C25H24O8	
Artonin E; 4'''-Hydroxy[c]			C25H24O8	
Atramycin A; 6-Deoxy[c]			C25H24O8	
Curcumin; Di-Ac[c]			C25H24O8	
9,10-Dihydro-3,9,10-trihydroxy-8,8-dimethyl-2-phenyl-4H,8H-benzo[1,2-b:3,4-b']dipyran-4-one; (9R*,10R*)-form, 3-Me ether, 9,10-di-Ac[c]			C25H24O8	
9,10-Dihydro-5,9,10-trihydroxy-8,8-dimethyl-2-phenyl-4H,8H-benzo[1,2-b:3,4-b']dipyran-4-one; (9R*,10R*)-form, 5-Me ether, 9,10-di-Ac[c]			C25H24O8	
Epimedokoreanin A[c]			C25H24O8	
Maackolin[c]			C25H24O8	
Propolisbenzofuran A[c]			C25H24O8	
513.084279	490.095049	490.090148	490.09995	0
528.0473004	505.0580704	505.0530198	505.063121	0
565.2863106	542.2970806	542.2916576	542.3025036	0

[a]m/z = mass to charge ratio of molecular ion fragments identified by the mass spectrometer
[b]ppm = parts per million
[c]also matched at 5 ppm and 2 ppm

Table 4.5. Chapman & Hall database-searched mass ranges and number of putative compound matches for Compound X based on m/z peaks obtained from LC/MS/MS and FTMS.

Modality Used	m/z[a]	Accuracy (ppm[b])	Low end of searched range (molar mass)	High end of searched range (molar mass)	# of database matches
LC/MS/MS[c]	146.073	1000	146.9332	147.2274	173
LC/MS/MS	157.0697	1000	157.9189	158.2351	112
LC/MS/MS	315.1263	500	315.9755	316.2916	110
LC/MS/MS	337.1211	50	338.1115	338.1453	149
LC/MS/MS	473.2008	100	474.1607	474.2555	71
LC/MS/MS	517.161	1000	517.6501	518.6864	214
FTMS[d]	317.0958	700	315.8672	316.3098	130
FTMS	475.137	250	474.0112	474.2482	213
FTMS	513.0843	700	511.7185	512.4355	16

[a]m/z = mass to charge ratio of molecular ion fragments identified by the mass spectrometer
[b]ppm = parts per million
[c]LC/MS/MS = liquid chromatography with tandem mass spectrometry
[d]FTMS = Fourier transform mass spectrometry

5. DISCUSSION

5.1 Characterization of Compound X

5.1.1 Compound X in diarrheal illness

Compound X was present in higher concentrations in serum from diarrheic calves than serum from healthy calves. The elevated Compound X in diarrheic calf serum may have arisen from increased absorption through the intestinal epithelium due to gastrointestinal bacterial production, epithelial barrier dysfunction, or in combination. Additional possible contributors to the increased serum Compound X include enhanced normal intestinal absorption, reduced renal clearance, and decreased metabolism of Compound X during diarrheal illness. Carbohydrate malabsorption during diarrheal illness can lead to proliferation of certain bacteria, namely *Lactobacillus spp.,* with consequent production of short-chain fatty acids and D-lactate (Ewaschuk, Naylor, Palmer et al., 2004). The elevated serum Compound X level in diarrheic calves matched the pattern for D-lactate observed by Ewaschuk (Ewaschuk, 2004). Conceivably, these bacteria may simultaneously produce other organic acids, one of which could be Compound X.

A mild positive correlation (Spearman's rho 0.289, $p = 0.003$) was observed between D-lactate and Compound X in diarrheic and healthy calf serum (Table 4.2). This relationship was not observed in calves that were infused with saline, HCl, L-lactic acid, or DL-lactic acid (Table 4.2). Furthermore, serum Compound X levels in acid-infused calves were very low, even in those that received the DL-lactic acid infusion (Figure 4.4). This suggests that some aspect of diarrheal illness, possibly the infectious agent, is involved in Compound X production or permeation through the intestinal wall. A limitation of the current study was that the role of hemoconcentration, due to dehydration, was not evaluated as a contributor to the increased serum Compound X levels in diarrheic illness.

Intestinal bacterial production of Compound X does not in itself explain the Compound X levels in biological fluids, since healthy calf feces contained large quantities of Compound X with little apparent permeation or absorption into the bloodstream (Figure 4.3). Intestinal permeability may be a significant contributory factor to the elevated Compound X levels in diarrheic calf serum compared to healthy calf serum. Ewaschuk (Ewaschuk, 2004) observed increased D-lactate absorption from the intestinal tract in diarrheic calves, indicating that small, microbial-produced molecules can be absorbed during diarrheal illness. Intestinal permeability

has been reported to increase during infection with *Cryptosporidium parvum*, rotavirus, and enteroaggregative *E. coli* (Kukuruzovic, 2002), as well as *Shigella* and enteropathogenic *E. coli* (Johansen, 1989). At least two of these, *Cryptosporidium parvum* and rotavirus, are within the four most significant causes of calf diarrhea (Foster, 2009), and, as such, could have accounted for a significant portion of the diarrheic calf cases. Unfortunately, the cause of the diarrhea was not determined in the studies from which the samples were obtained.

Compound X was relatively high in healthy calf feces compared to diarrheic feces and other biological fluids (Figure 4.3). The relatively low Compound X in diarrheic feces may, at least in part, be explained by a dilution effect in which abnormally large amounts of water are drawn into the intestinal lumen to produce diarrhea. However, this is opposite to the pattern observed by Ewaschuk (Ewaschuk, 2004), who reported increased fecal D-lactate during diarrheal illness. The large amount of Compound X in healthy feces suggests that it is a normal component of feces, such as an unabsorbed dietary component, a compound that is normally excreted in bile, or a bacterial metabolite. For instance, Compound X could be a di- or tripeptide, as oligopeptides have been reported to be present in digesta flowing at the ileum in calves (Montagne L, 2003). Oligopeptides have also been observed in calf plasma (Koeln, 1993); however they are present in only very small (micromolar) quantities in urine, owing to efficient renal reabsorption (Daniel, 2003). Since urinary Compound X levels were high in the current study, the likelihood of Compound X being an oligopeptide is very small.

The plausibility of Compound X originating from bile warrants consideration, since bile contains numerous organic compounds, including bile acids, phospholipids, cholesterol, bilirubin, proteins, peptides, and glutathione (Esteller, 2008). Many exogenous and endogenous compounds undergo enterohepatic circulation, and numerous metabolites are formed as these flow through the intestinal lumen, enterocytes, blood, hepatocytes, and biliary system (Esteller, 2008). The possibility remains that Compound X is one of these bile components or biliary component metabolites.

An inverse relationship (Spearman's rho = -0.682, $p < 0.0001$) (also see Figure 4.3) was observed between fecal Compound X and serum Compound X in healthy and diarrheic (combined) calves. Although fecal dilution of Compound X during diarrheal illness probably contributed to this relationship, it may also suggest that Compound X is an abundant, normal intestinal floral metabolite that is normally kept within the lumen by a healthy gut epithelium and

translocated across the epithelium during diarrheic illness. This hypothesis is backed by reports that intestinal permeability increases during infectious diarrhea (Isolauri, 1989; Zuckerman, 1993).

5.1.2 Compound X in D-lactic acidosis

Compound X was arguably related to diarrheic D-lactic acidosis more than D-lactic acidosis per se, as evidenced by differences between infused and diarrheic calves. The serum Compound X levels in DL-lactic acid-infused calves (median APAR = 1.86) were similar in magnitude to those of healthy calves (median APAR = 1.2), while the Compound X in diarrheic calves (median APAR = 4.8) was significantly greater than that of healthy calves. Additionally, the serum Compound X was significantly ($p<0.0001$) greater in diarrheic calves compared to DL-lactic acid-infused calves.

These relationships imply that Compound X is related to some aspect of the diarrheal illness, such as bacterial metabolism in the intestinal lumen. However, this is difficult to assess because no relationship was found between Compound X and D-lactate (produced by microbes) in feces and because Compound X was abundant in healthy calf feces and lower in diarrheic feces, which is opposite to the pattern observed by Ewaschuk (Ewaschuk, Naylor, Palmer et al., 2004) for fecal D-lactate in calves. Overall, comparisons with Ewaschuk's (Ewaschuk, 2004) data showed Compound X and D-lactate in healthy vs. diarrheic calves to have a similar pattern only for serum, while the feces and rumen fluid showed opposite patterns. The pattern for urine was also dissimilar, with Ewaschuk (Ewaschuk, 2004) reporting significantly increased D-lactate in diarrheic calves, while no significant difference was observed for urinary Compound X.

5.1.3 Compound X and neurological disturbance

Neurological disturbance associated with D-lactic acidosis, particularly after infusion of DL-lactic acid, in calves has been reported (Abeysekara, 2009), with the mechanism being related to D-lactate and not acidemia per se (Abeysekara, 2009). A low-moderate correlation (Spearman's rho = 0.399, $p = 0.01$) was found between serum Compound X and neurological disturbance score in DL-lactic acid-infused calves, but no correlation was observed between Compound X and D-lactate in serum or CSF from these calves. These findings imply that Compound X has some role in the neurological symptoms, but because they arose from analysis

of acid-infused calves that had only slightly elevated serum Compound X, the practical implications are uncertain.

Unfortunately, the data from diarrheic calves showed relationships that were opposite to those from acid-infused calves with regard to neurological disturbance and D-lactate. Specifically, serum Compound X and D-lactate were mildly correlated, while Compound X showed no correlation with neurologic disturbance in diarrheic calves. The major limitation of this analysis in diarrheic calves was the broad working definition of neurologic disturbance. An objectively-assigned neurological score for diarrheic calves would have improved the robustness of the analysis.

In order for Compound X to affect the nervous system, it would have to be present in the brain and cerebrospinal fluid. Abeysekara (Abeysekara, 2009) reported increased cerebrospinal fluid D-lactate levels that corresponded to neurological symptoms in DL-lactic acid-infused calves. Compound X was observed in cerebrospinal fluid from these calves, but the levels were not significantly different between DL-lactic acid-infused calves, saline-infused calves, and HCl-infused calves. These levels were also physiologically low compared to those of diarrheic calf serum. Nonetheless, a low-moderate positive correlation (Spearman's rho = 0.324, $p < 0.05$) existed between cerebrospinal fluid Compound X and neurological score, suggesting a possible link between the two.

5.1.4 Compound X in monogastric species

Compound X was present in serum from diarrheic piglets, foals, and human infants and the levels were significantly different from one another (Figure 4.5). Compound X was lowest in diarrheic piglet serum and highest in human infant serum. The differences could be in part due to the age differences between species. The diarrheic piglets were one to four days old, the diarrheic foals were less than 30 days old, the diarrheic calves were less than 45 days old, and the diarrheic human infants were less than three years old. Also, age- and species-specific susceptibilities to certain infectious causes of diarrhea may have differentially impacted the intestinal luminal environment. For example, the major causes of piglet diarrhea have been reported to be ETEC and rotavirus (Lecce, 1986), while foal diarrhea is commonly caused by *Clostridium perfringens, Clostridium difficile*, *Salmonella typhimurium,* and rotavirus (Magdesian, 2005). Calf diarrhea, on the other hand, is most often caused by enterotoxigenic

ETEC, *Cryptosporidium parvum*, coronavirus, and rotavirus (Foster, 2009). Differences in animal husbandry, specifically the clean piglet housing conditions and piglet treatment with antibiotics, could also affect Compound X by altering the intestinal flora. It is unknown if or how these factors affected Compound X, but it is not possible to rule out their influence.

Ewaschuk (Ewaschuk, 2004) reported elevated anion gap in diarrheic human infant serum with low (1-3 mmol/L) levels of L-LA and non-detectable levels of D-LA. It was suggested that another organic acid may be responsible for the elevated anion gap (Ewaschuk, 2004). If Compound X is an organic acid, it could help explain the elevated anion gap in diarrheic human infants. Indeed, Compound X was high in diarrheic human infant serum and was significantly higher than serum from diarrheic calves, foals, and piglets (Figure 4.5).

5.2 Efforts toward identifying Compound X

Compound X was hypothesized to be an organic acid, since it was first observed during HPLC using an organic acid-separating column and since it correlated with the anion gap (Omole, 1999). Compound X was elevated in serum from calves with diarrheal illness, a time during which many calves are neurologically depressed, raising the possibility that Compound X is a neurotoxin that contributes to neurological symptoms. Data from acid-infusion trials (Abeysekara, 2009) indicated that the neurologic disturbance was most pronounced during DL-lactic acid infusion. Intriguingly, the Compound X concentration was highest, albeit non-significantly, in CSF during DL-lactic acid infusion compared to other acid infusions, weakly supporting the hypothesis that Compound X contributes to neurological symptoms. However, lack of a clear relationship between Compound X and neurological dysfunction makes it unlikely that Compound X is a neurotoxin.

5.2.1 HPLC retention times for selected molecules

One way to identify an unknown compound, such as Compound X, is to check the retention times (RT) of possible compounds against that of Compound X using HPLC. A major limitation of this method is that the number of possible compounds could be very large, and the time and financial costs are prohibitive. Also, HPLC has poor specificity in that many compounds have similar RT's (Dalluge et al., 2002). This implies that Compound X could actually be a mixture of compounds that elute from the HPLC machine simultaneously. Thus,

even if a putative compound had a RT similar to Compound X, the identity of Compound X would need to be verified using more specific techniques.

Despite the limitations of this approach, the RT's of a small number of possible compounds were tested against Compound X. Although the data relating Compound X to D-lactic acidosis and neurologic symptoms was contradictory, there is a small possibility that Compound X depresses the central nervous system (CNS). The possibility that Compound X was an inhibitory neurotransmitter, GABA, was explored. HPLC analysis of CSF spiked with GABA failed to detect the compound using the described conditions (Figure 5.1). This indicates that Compound X is not likely GABA.

One of the putative matches arising from further LC/MS/MS was the amino acid neurotransmitter glutamic acid. Glutamic acid is the most abundant excitatory neurotransmitter in the mammalian CNS (Garattini, 2000). It is also the precursor for GABA, an inhibitory neurotransmitter. HPLC analysis of cerebrospinal fluid spiked with glutamic acid failed to detect the compound using the described conditions (Figure 5.2). This indicates that Compound X is not likely glutamic acid. Further evidence against Compound X being glutamic acid comes from the very high levels observed in healthy calf feces. Enterocytes have been reported to readily absorb and subsequently metabolize or sequester a large portion of the glutamate presented to the small intestine (Blachier, Boutry, Bos, & Tomé, 2009). On the contrary, significant amounts of protein and peptides from exogenous and endogenous sources have been observed in the colon, and these must be metabolized by bacteria, since there is little to no transfer of amino acids from the colonic lumen into portal blood (Blachier et al., 2009).

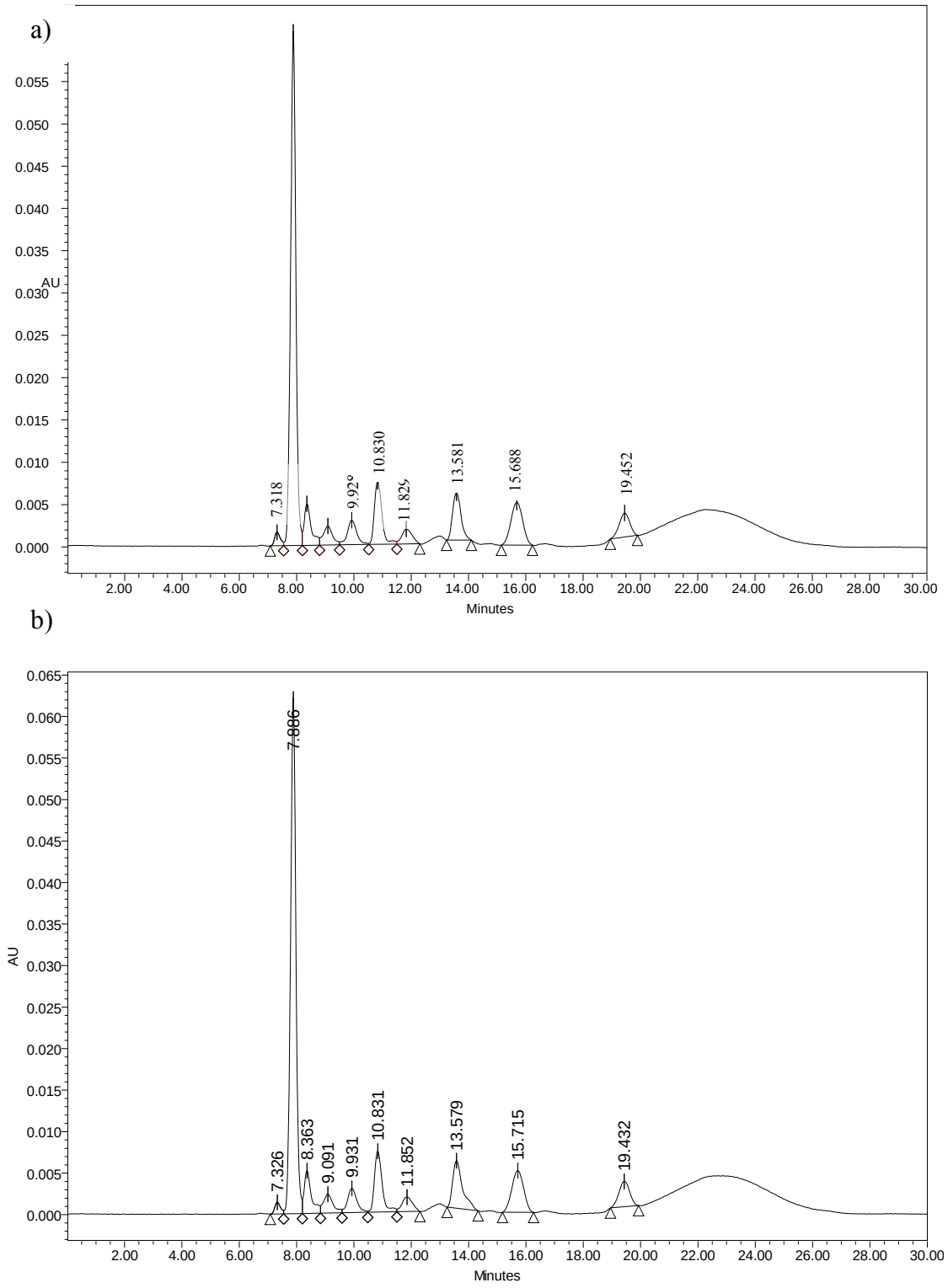
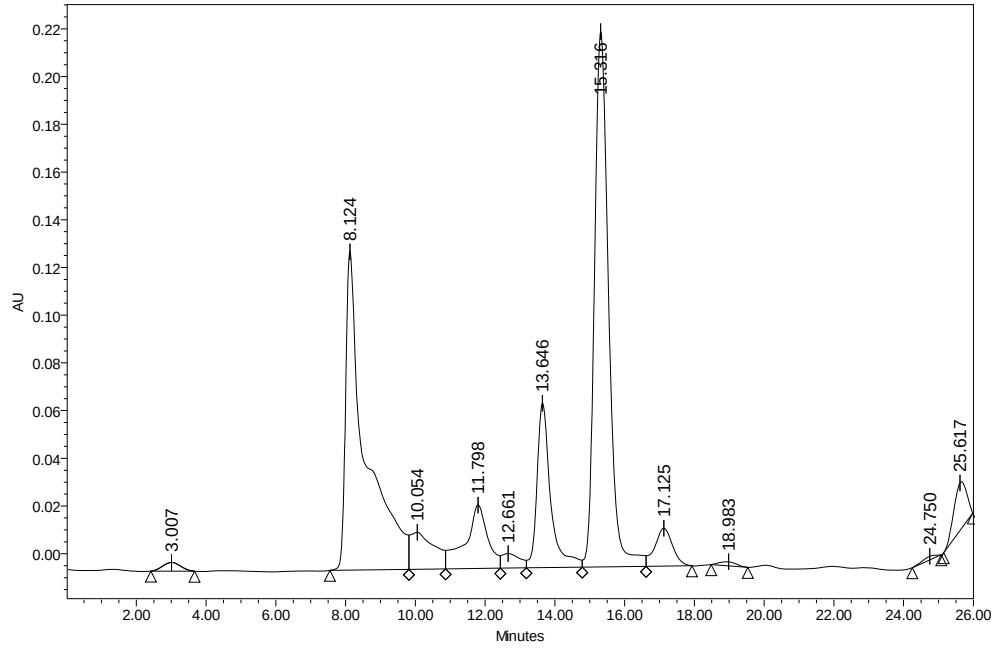


Figure 5.1. HPLC chromatograms showing a) healthy calf CSF and b) healthy calf CSF spiked with 50 mM gamma-amino butyric acid (GABA). GABA was not detected using the described HPLC conditions.

a)



b)

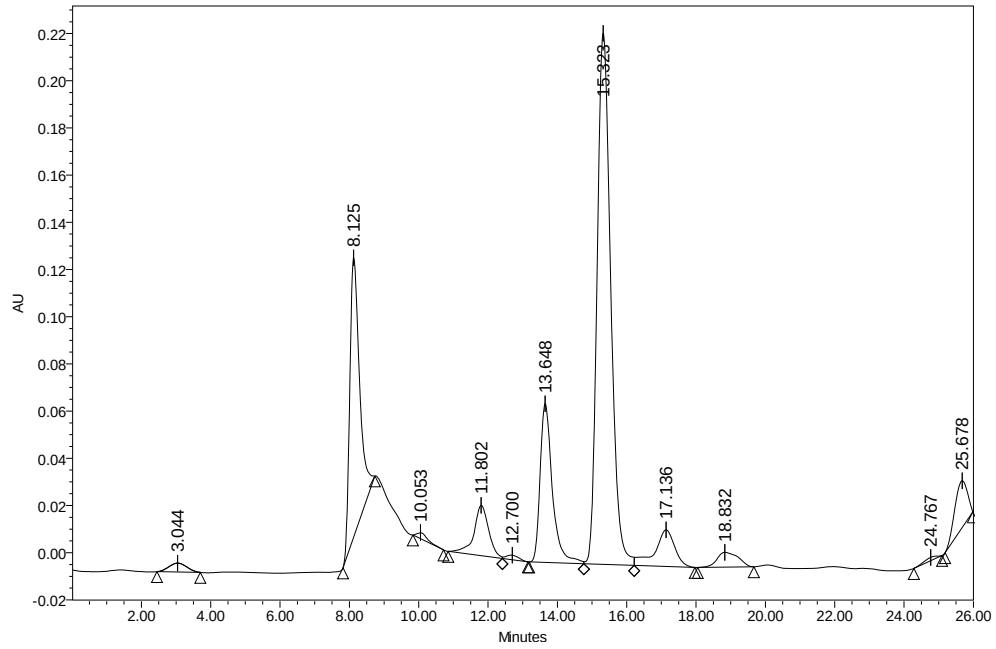


Figure 5.2. HPLC chromatogram showing a) diarrheic calf urine and b) diarrheic calf urine spiked with 25 mM glutamic acid. Glutamic acid had a retention time of 18.8 minutes, compared to Compound X's retention time of 15.3 minutes.

5.2.2 Limitations of gas chromatography for identifying Compound X

Gas chromatography (GC) of a typical urine sample showed a small unknown peak at 48.05 minutes, which closely matched the retention time (48.18 minutes) for the very large peak observed from the Compound X fraction. Unfortunately this peak was not identified with the database searches used.

The Compound X fraction was collected using HPLC, which implies that the fraction may have contained several different compounds that elute simultaneously from the HPLC column. Therefore, although the observed gas chromatograph peak was assumed to be Compound X, the possibility exists that Compound X may not have produced a peak and that the observed peak was another component of the fraction.

Many compounds can go undetected by GC, so derivatization (using trimethylsilylation) is used to reduce polarity and increase volatility of the compound of interest (Regis Technologies, 2000). Trimethylsilylation derivatizes alcohol, phenol, carboxyl, amine, and amide groups (Regis Technologies, 2000). Therefore, Compound X would have to lack these functional groups to avoid derivatization, which is possible but unlikely. On the other hand, Compound X may be a complex molecule containing several functional groups. In this case, the addition of numerous trimethylsilyl groups could cause either the Compound X molecule to become too large to pass through the GC column or the corresponding m/z ratio to exceed the mass range of the mass spectrometer detector (Halket, Waterman, Przyborowska, Patel, Fraser, & Bramley, 2004). As a result of these limitations, other techniques are required to identify Compound X.

5.2.3 Limitations of mass spectrometry for identifying Compound X

MS/MS and Fourier transform mass spectrometry FTMS did not provide a definitive identity of Compound X. Several factors may have contributed to this. One factor is that Compound X may be a mixture of compounds rather than a single compound. This is a limitation of the primary technique, HPLC, which is not necessarily specific enough to separate all of the individual components of a biological sample. Since fractions were collected and pooled using HPLC prior to MS/MS and FTMS, there may have been several compounds entering the mass spectrometers, which complicates the analysis. If this is the case, it would help explain the fact that several m/z peaks appeared on the spectrum from FTMS.

Another potential reason for Compound X not being identified by mass spectrometry is that Compound X may not have efficiently ionized under the conditions used. Ionization is a fundamentally essential step in mass spectrometric analysis, since the mass analyzer, by design, separates ions according to their mass to charge ratio (Van Bramer, 1998). Without ionization, a molecule will not be detected by the mass spectrometer's detector. When liquid chromatography precedes mass spectrometry, the likelihood is great that several compounds co-elute and therefore enter the mass spectrometer simultaneously. As the solution stream enters the mass spectrometer, the compound of interest may co-precipitate out of solution with non-volatile components, or it may be non-volatile itself and remain in the liquid portion of the stream and collect on the interface plate of the mass spectrometer (King, 2000). In either case, the molecule will fail to be ionized.

A final limitation of the FTMS was that the lowest observed m/z ratio was 257, corresponding to a molecular weight of 256 u. This is higher than many common organic acids in biological systems. For instance, the molar masses of larger common organic acids such as uric acid and citric acid are 168 and 192 g/mol, respectively. It may be beneficial in the future to explore m/z ratios in the 90 – 200 range using FTMS to determine whether or not there are any putative matches for Compound X in this range.

5.2.4 Future efforts toward identifying Compound X

Compound X was present in healthy, sick, and acid-infused animals. Further clues as to its origin could be obtained from HPLC analysis to search for Compound X in the milk replacer fed to calves or in the acid and saline infusates used in Abeysekara's (Abeysekara, 2009) studies.

Importantly, Compound X may be a collection of compounds that elute around the same time using the HPLC conditions described in section 3.4. Employment of a technique, such as ultra performance liquid chromatography (UPLC), could resolve the individual peaks that comprise the Compound X peak obtained with HPLC. UPLC systems use columns with smaller packing particle size, which increases the efficiency and resolving power of the system (Swartz, 2005).

Further study of Compound X using mass spectrometry may be warranted, particularly if the Compound X peak can be further resolved using UPLC. FTMS is advantageous over MS/MS with respect to resolution, precision, and accuracy (Zhang, McCombie, Guenat, & Knochenmuss,

2005). Since the lowest observed m/z from FTMS analysis was 257 u and many organic acids have masses well below this, future FTMS analysis should focus on a lower (e.g., 50 – 250) m/z ratio range.

Future efforts toward identifying Compound X may do well to make use of nuclear magnetic resonance (NMR) spectrometry. A major strength of NMR is that it provides information about functional groups within molecules (Mitchell, 2007). This would be useful in that carboxylic acid groups or amino groups could be identified to provide clues to the identity of Compound X. Previously, the sample requirements for NMR analysis introduced challenges, as samples were required to be isolated, purified, and of relatively large volume (Chary, 2008.). However, NMR has been refined into a highly sensitive technique, and it is now common to couple HPLC to NMR spectrometry (Mitchell, 2007). Although costly deuterated solvents are normally used for NMR, these can be kept to a minimum by using techniques designed to suppress confounding solvent signals (Mitchell, 2007). As such, NMR appears to be a reasonable next step for determining the identity of Compound X.

6. CONCLUSIONS

Compound X was determined to be ubiquitous in the biological fluids studied, implying that it is a normal biological compound. Fecal Compound X levels were high in healthy calves, suggesting that Compound X is produced by intestinal bacteria. The elevated serum Compound X levels in diarrheic calves may have been caused by absorption of Compound X from the intestinal lumen into the bloodstream. As serum Compound X increases in diarrheal illness, it may partly explain the neurological signs observed in diarrheic, D-lactic acidotic calves.

Compound X positively correlated with D-lactate in clinical cases and positively correlated with neurological score in experimentally infused calves, insinuating a potential role for Compound X in the neurological disturbance. Additionally, Compound X may be pertinent to human infants, since it was significantly elevated in serum from diarrheic infants in whom increased anion gap could not be explained by L- or D-lactate.

In light of the aforementioned findings, Compound X is worthy of further study to determine its identity. HPLC with mass spectrometry was unsuccessful in achieving this, but other biochemical techniques, such as NMR, may prove to be more effective in this regard. Also, techniques to separate the probable several components of the isolated Compound X HPLC peak may be required prior to further attempts to identify Compound X. Since identification of an unknown compound is not a trivial task, this might best be accomplished by seeking partnership with an institution or company that specializes in biochemical techniques used for molecular isolation, purification, and isolation.

REFERENCES

Abeysekara, S. (2009). D-lactic acid metabolism and control of acidosis (Doctoral dissertation). University of Saskatchewan, Saskatoon, Saskatchewan.

Abeysekara, A. W. A. S., Naylor, J. M., & Zello, G. A. (2006). Influence of acidity and lactates in blood and CSF on neurological disturbances (Abstract presentation, 13th Annual Life and Health Sciences Student Research Conference, University of Saskatchewan). Saskatoon, Saskatchewan.

Abeysekara, S., Naylor, J. M., Wassef, A. W., Isak, U., & Zello, G. A. (2007). D-lactic acid-induced neurotoxicity in a calf model. *American Journal of Physiology - Endocrinology & Metabolism, 293*(2), E558-65.

Acres, S. D. (1977). Acute undifferentiated neonatal diarrhea of beef calves: The prevalence of enterotoxigenic E. coli, reo-like (rota) virus and other enteropathogens in cow-calf herds. *The Canadian Veterinary Journal, 18*(5), 113.

Applied Biosystems. Retrieved December 5, 2009, from https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=600836&tab=TechSpec

Ayers, P., & Warrington, L. (2008). Diagnosis and treatment of simple acid-base disorders. *Nutrition in Clinical Practice, 23*(2), 122-127.

Azhar, S. S., & Beach, R. E. (2002). D-lactic acidosis in a diabetic patient with a short bowel. *Journal of the American Board of Family Practice, 15*(4), 316-318.

Baldi, F., Bianco, M. A., Nardone, G., Pilotto, A., & Zamparo, E. (2009). Focus on acute diarrhoeal disease. *World Journal of Gastroenterology, 15*(27), 3341-3348.

Beef Information Centre. (2007). *Canada's beef industry fast facts* Retrieved from http://albertabeef.org/res/cp-Fast%20Facts%20English%20Final%20Oct%2007.pdf

Bird, I. M. (1989). High performance liquid chromatography: Principles and clinical applications. *BMJ.British Medical Journal (Clinical Research Ed.), 299*(6702), 783.

Blachier, F., Boutry, C., Bos, C., & Tomé, D. (2009). Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. *The American Journal of Clinical Nutrition 90*(3), 814S-821S.

Brown, A. J. (2008). Fluid therapy in vomiting and diarrhea. *The Veterinary Clinics of North America.Small Animal Practice, 3*8(3), 653.

Chace, D. H. (2003). Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. *Clinical Chemistry, 4*9(11), 1797.

Chary, K. V. R. (2008.). In Govil G., MyiLibrary L. (Eds.), *NMR in biological systems: From molecules to huma*n. Dordrecht, Netherlands: Springer.

Checkley, W. (2008). Multi-country analysis of the effects of diarrhoea on childhood stunting. *International Journal of Epidemiology, 3*7(4), 816.

Clancy J. (2007). Short-term regulation of acid-base homeostasis of body fluids. *British Journal of Nursing, 1*6(16), 1016.

Colby College Chemistry. (1997). *Fragment finde*r. Maine, United States: Retrieved from http://www.colby.edu/chemistry/PChem/Fragment.html

Colby College Chemistry. (1998). *Formula finde*r. Maine, United States: Retrieved from http://www.colby.edu/chemistry/PChem/Formula.html

Dahlquist, N. R., Perrault, J., Callaway, C. W., & Jones, J. D. (1984). D-lactic acidosis and encephalopathy after jejunoileostomy: Response to overfeeding and to fasting in humans. *Mayo Clinic Proceedings, 5*9(3), 141-145.

Dalluge, J. J., Gort, S., Hobson, R., Selifonova, O., Amore, F., & Gokarn, R. (2002). Separation and identification of organic acid-coenzyme A thioesters using liquid chromatography/electrospray ionization-mass spectrometry. *Analytical & Bioanalytical Chemistry, 3*74(5), 835-840.

Daniel, H. (2003). An update on renal peptide transporters. *American Journal of Physiology.Renal, Fluid and Electrolyte Physiology, 28*4(5), F885.

Dubose, J. T. D. (2008). Chapter 48. acidosis and alkalosis. In A. S. Fauci, E. Braunwald, D. L. Kasper, S. L. Hauser, D. L. Longo, J. L. Jameson & J. Loscalzo (Eds.), *Harrison's principles of internal medicin*e (17th ed.,) Retrieved from http://www.accessmedicine.com/content.aspx?aID=2866352

Edwards, S. L. (2008). Pathophysiology of acid base balance: The theory practice relationship. *Intensive & Critical Care Nursing, 2*4(1), 28-38.

Esteller, A. (2008). Physiology of bile secretion. *Shi Jie Chang Wei Bing Xue Za Zhi, 1*4(37), 5641.

Ewaschuk, J. B., Naylor, J. M., Barabash, W. A., & Zello, G. A. (2004). High-performance liquid chromatographic assay of lactic, pyruvic and acetic acids and lactic acid stereoisomers in calf

feces, rumen fluid and urine. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences, 805*(2), 347-351.

Ewaschuk, J. B., Naylor, J. M., Chirino-Trejo, M., & Zello, G. A. (2004). Lactobacillus rhamnosus strain GG is a potential probiotic for calves. *Canadian Journal of Veterinary Research, 6*8(4), 249-253.

Ewaschuk, J. B., Naylor, J. M., Palmer, R., Whiting, S. J., & Zello, G. A. (2004). D-lactate production and excretion in diarrheic calves. *Journal of Veterinary Internal Medicine, 1*8(5), 744-747.

Ewaschuk, J. B., Naylor, J. M., & Zello, G. A. (2003). Anion gap correlates with serum D- and DL-lactate concentration in diarrheic neonatal calves. *Journal of Veterinary Internal Medicine, 1*7(6), 940-942.

Ewaschuk, J. B., Zello, G. A., Naylor, J. M., & Brocks, D. R. (2002). Metabolic acidosis: Separation methods and biological relevance of organic acids and lactic acid enantiomers. [review] [108 refs]. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences, 78*1(1-2), 39-56.

Ewaschuk, J. B. (2004). Mechanism and treatment of acidosis in neonatal diarrhea (Doctoral dissertation). University of Saskatchewan, Saskatoon, Saskatchewan.

Fayer, R. (2007). Prevalence of cryptosporidium species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. *Veterinary Parasitology, 145*(3), 260.

Fidkowski, C., & Helstrom, J. (2009). Diagnosing metabolic acidosis in the critically ill: Bridging the anion gap, Stewart, and base excess methods. *Canadian Journal of Anaesthesia, 5*6(3), 247-256.

Field, M. (2003). Intestinal ion transport and the pathophysiology of diarrhea. *Journal of Clinical Investigation, 11*1(7), 931.

Foster, D. M. (2009). Pathophysiology of diarrhea in calves. *The Veterinary Clinics of North America.Food Animal Practice, 25*(1), 13.

Gandhi, G. K. (2009). Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *Journal of Neurochemistry, 11*1(2), 522.

Garattini, S. (2000). Glutamic acid, twenty years later. *The Journal of Nutrition, 13*0(4), 901S.

Gauthier, P. M. (2002). Metabolic acidosis in the intensive care unit. *Critical Care Clinics, 1*8(2), 289.

Gibbs, M. E. (2008). Inhibition of astrocytic energy metabolism by D-lactate exposure impairs memory. *Neurochemistry International, 5*2(6), 1012.

Goens, S. D. (2002). The evolution of bovine viral diarrhea: A review. *The Canadian Veterinary Journal, 4*3(12), 946.

Gow S. (2005). The effect of treatment duration on weaning weights in a cow-calf herd with a protracted severe outbreak of diarrhea in calves. *The Canadian Veterinary Journal, 4*6(5), 418-23,425.

Grove-White, D. H. (1998). Monitoring and management of acidosis in calf diarrhoea. [review] [21 refs]. *Journal of the Royal Society of Medicine, 9*1(4), 195-198.

Grove-White, D. H., & White, D. G. (1993). Diagnosis and treatment of metabolic acidosis in calves: A field study. *Veterinary Record, 13*3(20), 499-501.

Gurevitch J. (1993). D-lactic acidosis: A treatable encephalopathy in pediatric patients. *Acta Pædiatrica, 8*2(1), 119.

Halket, J., Waterman, D., Przyborowska, A., Patel, R., Fraser, P., & Bramley, P. (2004). Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. *Journal of Experimental Botany, 56*(410), 219-243.

Hellard, M. E. (2003). Cost of community gastroenteritis. *Journal of Gastroenterology and Hepatology, 1*8(3), 322.

Herikstad, H. (2002). A population-based estimate of the burden of diarrhoeal illness in the United States: FoodNet, 1996-7. *Epidemiology and Infection, 12*9(1), 9.

Hoffmann, G. F. (1993). Physiology and pathophysiology of organic acids in cerebrospinal fluid. *Journal of Inherited Metabolic Disease, 1*6(4), 648.

Hoffmann, G., Aramaki, S., Blum-Hoffmann, E., Nyhan, W. L., & Sweetman, L. (1989). Quantitative analysis for organic acids in biological samples: Batch isolation followed by gas chromatographic-mass spectrometric analysis. *Clinical Chemistry, 3*5(4), 587-595.

Holland, R. E. (1990). Some infectious causes of diarrhea in young farm animals. *Clinical Microbiology Reviews,* 3(4), 345.

Imagawa, H., Sekiguchi, K., Anzai, T., Fukunaga, Y., Kanemaru, T., Ohishi, H., Higuchi, T., & Kamada, M. (1991). Epidemiology of equine rotavirus infection among foals in the breeding region. *Journal of Veterinary Medical Science, 5*3(6), 1079-1080.

Isolauri, E. (1989). Intestinal permeability changes in acute gastroenteritis: Effects of clinical factors and nutritional management. *Journal of Pediatric Gastroenterology and Nutrition,* 8(4), 466.

Johansen, K. (1989). Intestinal permeability assessed with polyethylene glycols in children with diarrhea due to rotavirus and common bacterial pathogens in a developing community. *Journal of Pediatric Gastroenterology and Nutrition,* 9(3), 307.

Kaae, J., & de Morais, H. A. (2008). Anion gap and strong ion gap: A quick reference. *Veterinary Clinics of North America - Small Animal Practice, 3*8(3), 443-447.

Kasari, T. R. (1999). Metabolic acidosis in calves. [review] [35 refs]. *Veterinary Clinics of North America - Food Animal Practice, 1*5(3), 473-486.

Kawana S. (2008). Improvement of sample throughput using fast gas chromatography mass-spectrometry for biochemical diagnosis of organic acid disorders. *Clinica Chimica Acta, 39*2(1-2), 34.

Kellum, J. A. (2005). Determinants of plasma acid-base balance. *Critical Care Clinics, 2*1(2), 329.

Keppie, N. J. (2005). A retrospective study of the diagnoses and survival of elk admitted to a large animal referral clinic. *The Canadian Veterinary Journal, 4*6(4), 325.

King, R. (2000). Mechanistic investigation of ionization suppression in electrospray ionization. *Journal of the American Society for Mass Spectrometry, 1*1(11), 942.

Koeln, L. L. (1993). Amino acid flux across the gastrointestinal tract and liver of calves. *Journal of Dairy Science, 7*6(8), 2275.

Kosek, M. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *World Health Organization.Bulletin of the World Health Organization, 8*1(3), 197.

Kukuruzovic, R. (2002). Enteric pathogens, intestinal permeability and nitric oxide production in acute gastroenteritis. *The Pediatric Infectious Disease Journal, 2*1(8), 730.

Lawson, A. M. (1976). Urinary organic acids in man. I. normal patterns. *Clinical Chemistry, 22*(8), 1283.

LeBlond, R. F., Brown, D. D., & DeGowin, R. L. (2009). Chapter 18: Common laboratory tests. In L. The McGraw-Hill Companies (Ed.), *DeGowin's diagnostic examination* (9th ed.)

Lecce, J. G. (1986). Diarrhea: The nemesis of the artificially reared, early weaned piglet and a strategy for defense. *Journal of Animal Science, 63*(4), 1307.

Lehotay, D. C. (1995). Organic acidurias and related abnormalities. *Critical Reviews in Clinical Laboratory Sciences, 32*(4), 377.

Linke, H. A., & Moss, S. J. (1992). Quantitation of carbohydrate sweeteners and organic acids in human oral fluid using HPLC analysis. *Zeitschrift Fur Ernahrungswissenschaft, 31*(2), 147-154.

Lord, R. S. (2008). Clinical applications of urinary organic acids. part I: Detoxification markers. *Alternative Medicine Review, 13*(3), 205.

Lorenz, I., & Vogt, S. (2006). Investigations on the association of D-lactate blood concentrations with the outcome of therapy of acidosis, and with posture and demeanour in young calves with diarrhoea. *Journal of Veterinary Medicine - Series A, 53*(9), 490-494.

Lough, W. J., & Wainer, I. W. (1996). *High performance liquid chromatography :Fundamental principles and practice* (1st ed.). London: Blackie Academic & Professional.

Luft, D. (1983). Definition of clinically relevant lactic acidosis in patients with internal diseases. *American Journal of Clinical Pathology, 80*(4), 484.

Magdesian, K. G. (2005). Neonatal foal diarrhea. *The Veterinary Clinics of North America.Equine Practice, 21*(2), 295.

Marks, S. L. (2003). Bacterial-associated diarrhea in the dog: A critical appraisal. *The Veterinary Clinics of North America.Small Animal Practice, 33*(5), 1029.

Marsden, D. L. (1992). Neurological diseases in disorders of organic acids. *Current Opinion in Neurology and Neurosurgery, 5*(3), 349.

Mitchell, T. N. (2007). In Costisella B., SpringerLink (Online service)(Eds.), *NMR - from spectra to structures: An experimental approach* (Second Revised and Expanded Edition. ed.). Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg.

Moeser, A. J. (2007a). Mechanisms of porcine diarrheal disease. *Journal of the American Veterinary Medical Association, 231*(1), 56.

Moeser, A. J. (2007b). Mechanisms of porcine diarrheal disease. *Journal of the American Veterinary Medical Association, 231*(1), 56.

Montagne L. (2003). Influence of dietary protein level and source on the course of protein digestion along the small intestine of the veal calf. *Journal of Dairy Science, 86*(3), 934.

Morris, C. G., & Low, J. (2008a). Metabolic acidosis in the critically ill: Part 1. classification and pathophysiology. *Anaesthesia, 63*(3), 294-301.

Morris, C. G., & Low, J. (2008b). Metabolic acidosis in the critically ill: Part 2. causes and treatment. *Anaesthesia, 63*(4), 396-411.

Nakagawa, M., Suzuki, K., & Taguchi, K. (2007). Relationship between depression score and acid-base status in Japanese black calves with diarrhea. *Journal of Veterinary Medical Science, 69*(5), 549-552.

Narula, R. K., El Shafei, A., Ramaiah, D., & Schmitz, P. G. (2000). D-lactic acidosis 23 years after jejuno-ileal bypass. *American Journal of Kidney Diseases, 36*(2), E9.

Oh, M. S., Uribarri, J., Alveranga, D., Lazar, I., Bazilinski, N., & Carroll, H. J. (1985). Metabolic utilization and renal handling of D-lactate in men. *Metabolism: Clinical & Experimental, 34*(7), 621-625.

Oldendorf, W. H. (1973). Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *American Journal of Physiology, 224*(6), 1450.

Omole, O. O. (1999). Lactic acidosis in neonatal calf diarrhea (Master of Science thesis). University of Saskatchewan, Saskatoon, Saskatchewan.

Omole, O. O., Brocks, D. R., Nappert, G., Naylor, J. M., & Zello, G. A. (1999). High-performance liquid chromatographic assay of (+/-)-lactic acid and its enantiomers in calf serum. *Journal of Chromatography.B, Biomedical Sciences & Applications, 727*(1-2), 23-29.

Omole, O. O., Nappert, G., Naylor, J. M., & Zello, G. A. (2001). Both L- and D-lactate contribute to metabolic acidosis in diarrheic calves. *Journal of Nutrition, 131*(8), 2128-2131.

Ott, S. (1993). *Dairy heifer morbidity, mortality, and health management focusing on preweaned heifers.* Retrieved 10/25, 2009, from http://purl.umn.edu/32756

Parashar, U. D., Bresee, J. S., & Glass, R. I. (2003). The global burden of diarrhoeal disease in children. *Bulletin of the World Health Organization, 8*1(4), 236.

Petersen, C. (2005). D-lactic acidosis. [review] [53 refs]. *Nutrition in Clinical Practice, 2*0(6), 634-645.

Regis Technologies. (2000). *GC Derivatization*. Retrieved from http://www.registech.com/Library/gcderrev.pdf

Reinnig, M. C. (2008). Characterization of selected organic compound classes in secondary organic aerosol from biogenic VOCs by HPLC/MSn. *Analytical and Bioanalytical Chemistry, 39*1(1), 171.

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science, 8*2(4), 632.

Scallan, E., Majowicz, S. E., Hall, G., Banerjee, A., Bowman, C. L., Daly, L., Jones, T., Kirk, M. D., Fitzgerald, M., & Angulo, F. J. (2005). Prevalence of diarrhoea in the community in Australia, Ccanada, Ireland, and the United States. *International Journal of Epidemiology, 3*4(2), 454-460.

Scott, R. P. (2003). The UV detectors. In *Liquid chromatograph*y. Retrieved from http://www.chromatography-online.org/HPLC-Detectors/UV/rs37.html

Sellin, J. H. (2001). The pathophysiology of diarrhea. *Clinical Transplantation, 1*5(4), 2.

Swartz, M.E. (2005). *Ultra performance liquid chromatography: An introduction*. Retrieved May 5, 2010 from http://chromatographyonline.findanalytichem.com/lcgc/data/articlestandard/lcgc/242005/164646/article.pdf

Taylor & Francis Group. (2010). *Dictionary of natural product*s Retrieved from http://dnp.chemnetbase.com/dictionary-search.do?method=view&id=7517&si=

Taylor, L. F. (1997). Performance, survival, necropsy, and virological findings from calves persistently infected with the bovine viral diarrhea virus originating from a single Saskatchewan beef herd. *The Canadian Veterinary Journal, 3*8(1), 29.

The United Nations Children's Fund (UNICEF)/World Health Organization (WHO). (2009). *Diarrhoea: Why children are still dying and what can be don*e Retrieved from http://whqlibdoc.who.int/publications/2009/9789241598415_eng.pdf

Thurn, J. R. (1985). D-lactate encephalopathy. *The American Journal of Medicine, 79,* 717-721.

Tiwari, A., Vanleeuwen, J. A., Dohoo, I. R., Keefe, G. P., Haddad, J. P., Tremblay, R., Scott, H. M., & Whiting, T. (2007). Production effects of pathogens causing bovine leukosis, bovine viral diarrhea, paratuberculosis, and neosporosis. *Journal of Dairy Science, 9*0(2), 659-669.

Tiwari, A., VanLeeuwen, J. A., Dohoo, I. R., Stryhn, H., Keefe, G. P., & Haddad, J. P. (2005). Effects of seropositivity for bovine leukemia virus, bovine viral diarrhoea virus,

mycobacterium avium subspecies paratuberculosis, and neospora caninum on culling in dairy cattle in four Canadian provinces. *Veterinary Microbiology, 109*(3-4), 147-158.

Tomkins, A., & Watson, F. (1989). *Malnutrition and infection − A review − nutrition policy discussion paper no.* 5. Switzerland: United Nations. Retrieved from http://www.unscn.org/layout/modules/resources/files/Policy_paper_No_5.pdf

Tsao, G. T. (1999). Production of multifunctional organic acids from renewable resources. *Advances in Biochemical Engineering/Biotechnology, 65*, 243.

Tubbs, P. K. (1965). The metabolism of D-alpha-hydroxy acids in animal tissues. *Annals of the New York Academy of Sciences, 119*(3), 920.

Uchida, H., Yamamoto, H., Kisaki, Y., Fujino, J., Ishimaru, Y., & Ikeda, H. (2004). D-lactic acidosis in short-bowel syndrome managed with antibiotics and probiotics. *Journal of Pediatric Surgery, 39*(4), 634-636.

United States Department of Agriculture. (2007). *Cattle and calves nonpredator death loss in the United States, 2005*

United States Department of Agriculture Economic Research Service. (2009). *U.S. beef and cattle industry: Background statistics and information*. Retrieved 2010, 2009, from http://www.ers.usda.gov/news/bsecoverage.htm

Uribarri, J., Oh, M. S., & Carroll, H. J. (1998). D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. [review] [72 refs]. *Medicine, 77*(2), 73-82.

USDA. (1991-2007). *Dairy 2007, part II: Changes in the United States dairy industr*y. Fort Collins, CO: USDA-APHIS-VS, CEAH. Retrieved from http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/dairy07/Dairy2007_PartII.pdf

Valle, P. S. (2005). Ten years of bovine virus diarrhoea virus (BVDV) control in Norway: A cost-benefit analysis. *Preventive Veterinary Medicine, 72*(1-2), 189-207 discussion215.

Van Bramer, S. (1998). *An introduction to mass spectrometry.* Retrieved from http://science.widener.edu/svb/massspec/massspec.pdf

Widdowson, M. A. (2005). Rotavirus disease and its prevention. *Current Opinion in Gastroenterology, 21*(1), 26.

Wong, C. K. (2008). Features of ciguatera fish poisoning cases in Hong Kong 2004-2007. *Biomedical and Environmental Sciences, 21*(6), 521.

World population data sheet 2009. (2009). *Annual Report of the Population Reference Bureau.* Washington, DC, USA.

Yesilbag K. (2007). Aetiological role of viruses in puppies with diarrhoea. *The Veterinary Record, 16*1(5), 169.

Zhang Y. (2000). Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and cryptosporidium. diarrhea working group, Peru. *Journal of Pediatric Gastroenterology and Nutrition, 3*1(1), 16.

Zhang, D. L., Jiang, Z. W., Jiang, J., Cao, B., & Li, J. S. (2003). D-lactic acidosis secondary to short bowel syndrome. *Postgraduate Medical Journal, 7*9(928), 110-112.

Zhang, J., McCombie, G., Guenat, C., & Knochenmuss, R. (2005). FT-ICR mass spectrometry in the drug discovery process. *Drug Discovery Today, 10*(9), 635-642.

Zoran, D. L. (2008). Nutritional management of feline gastrointestinal diseases. *Topics in Companion Animal Medicine, 2*3(4), 200.

Zuckerman, M. J. (1993). Intestinal permeability to [51Cr]EDTA in infectious diarrhea. *Digestive Diseases and Sciences, 3*8(9), 1651.

APPENDICES

Table A.1. Putative matches for Compound X from the Chapman & Hall database based on m/z ratios obtained from LC/MS/MS analysis of pooled Compound X fractions from HPLC of calf urine.

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
146.073	**1000**		
		Actinidine	C10H13N
		Actinidine; (R)-form	C10H13N
		Actinidine; (S)-form	C10H13N
		Actinidine; (±)-form	C10H13N
		Albizziine	C4H9N3O3
		Albizziine; (R)-form	C4H9N3O3
		Albizziine; (S)-form	C4H9N3O3
		Albizziine; (±)-form	C4H9N3O3
		2-Amino-3-hydroxybutanoic acid; (2R,3S)-form, N-Formyl	C5H9NO4
		2-Amino-3-hydroxybutanoic acid; (2S,3R)-form, N-Formyl	C5H9NO4
		2-Amino-4-hydroxybutanoic acid; (S)-form, Et ether	C6H13NO3
		2-Amino-4-hydroxy-3,3-dimethylbutanoic acid	C6H13NO3
		2-Amino-4-hydroxy-3,3-dimethylbutanoic acid; (±)-form	C6H13NO3
		2-Amino-5-hydroxyhexanoic acid	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid; (2S,3R)-form	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid; (2S,3S)-form	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid; (2RS,3RS)-form	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid; (2RS,3SR)-form	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid; (2R,3R)-form	C6H13NO3
		2-Amino-3-hydroxy-4-methylpentanoic acid; (2R,3S)-form	C6H13NO3
		2-Amino-4-hydroxy-3-methylpentanoic acid	C6H13NO3
		2-Amino-4-hydroxy-3-methylpentanoic acid; (2R,3R,4R)-form	C6H13NO3
		2-Amino-4-hydroxy-3-methylpentanoic acid; (2S,3R,4S)-form	C6H13NO3
		2-Amino-4-hydroxy-3-methylpentanoic acid; (2R,3R,4S)-form	C6H13NO3
		2-Amino-4-hydroxy-3-methylpentanoic acid; (2S,3R,4R)-form	C6H13NO3
		2-Amino-4-hydroxy-3-methylpentanoic acid; (2S,3S,4R)-form	C6H13NO3

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
146.073 (continued)	**1000**	2-Amino-4-hydroxy-4-methylpentanoic acid	C6H13NO3
		2-Amino-4-hydroxy-4-methylpentanoic acid; (S)-form	C6H13NO3
		2-Amino-4-hydroxy-4-methylpentanoic acid; (±)-form	C6H13NO3
		2-Amino-5-hydroxy-4-oxopentanoic acid	C5H9NO4
		2-Amino-5-hydroxy-4-oxopentanoic acid; (S)-form	C5H9NO4
		2-Amino-5-hydroxy-4-oxopentanoic acid; (±)-form	C5H9NO4
		2-Amino-3-methylbutanedioic acid	C5H9NO4
		2-Amino-3-methylbutanedioic acid; (2R,3R)-form	C5H9NO4
		2-Amino-3-methylbutanedioic acid; (2S,3S)-form	C5H9NO4
		2-Amino-3-methylbutanedioic acid; (2RS,3RS)-form	C5H9NO4
		2-Amino-3-methylbutanedioic acid; (2RS,3SR)-form	C5H9NO4
		2-Amino-3-methylbutanedioic acid; (2S,3R)-form	C5H9NO4
		2-Amino-3-methylbutanedioic acid; (2S,3S)-form, N-Hydroxy	C5H9NO4
		3-Aminopentanedioic acid, 9CI	C5H9NO4
		1-(2-Aminophenyl)-2-propen-1-one	C9H9NO
		3-Aminopropanal, 9CI; Di-Et acetal	C7H17NO2
		3-Amino-2,3,6-trideoxy-arabino-hexose	C6H13NO3
		3-Amino-2,3,6-trideoxy-arabino-hexose; D-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-arabino-hexose; a-D-Pyranose-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-arabino-hexose; ß-D-Pyranose-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-arabino-hexose; L-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-arabino-hexose; a-L-Pyranose-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-lyxo-hexose	C6H13NO3
		3-Amino-2,3,6-trideoxy-lyxo-hexose; D-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-lyxo-hexose; a-D-Pyranose-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-lyxo-hexose; L-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-lyxo-hexose; a-L-Pyranose-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-lyxo-hexose; DL-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-ribo-hexose	C6H13NO3
		3-Amino-2,3,6-trideoxy-ribo-hexose; L-form	C6H13NO3
		3-Amino-2,3,6-trideoxy-ribo-hexose; a-L-form	C6H13NO3
		4-Amino-2,4,6-trideoxy-arabino-hexose	C6H13NO3
		4-Amino-2,4,6-trideoxy-arabino-hexose; L-form	C6H13NO3
		4-Amino-2,4,6-trideoxy-arabino-hexose; a-L-Pyranose-form	C6H13NO3
		4-Amino-2,4,6-trideoxy-arabino-hexose; ß-L-Pyranose-form	C6H13NO3
		4-Amino-2,4,6-trideoxy-arabino-hexose; ß-DL-Pyranose-form	C6H13NO3
		Aspartic acid, USAN, INN; (S)-form, 1-Hydrazide	C4H9N3O3
		Aspartic acid, USAN, INN; (R)-form, 1-Me ester	C5H9NO4
		Aspartic acid, USAN, INN; (S)-form, 1-Me ester	C5H9NO4
		Aspartic acid, USAN, INN; (S)-form, 4-Me ester	C5H9NO4
		Aspartic acid, USAN, INN; (±)-form, 4-Me ester	C5H9NO4
		Aspartic acid, USAN, INN; (R)-form, N-Me	C5H9NO4
		Aspartic acid, USAN, INN; (S)-form, N-Me	C5H9NO4

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
146.073 (continued)	**1000**	Aspartic acid, USAN, INN; (±)-form, N-Me	C5H9NO4
		Benzoic acid, USAN, 9CI; Azide	C7H5N3O
		N-(Carboxymethyl)alanine, 9CI	C5H9NO4
		N-(Carboxymethyl)alanine, 9CI; (S)-form	C5H9NO4
		3-(Carboxymethylamino)propanoic acid	C5H9NO4
		Coelobillardierine; (±)-form, Ketone	C9H9NO
		Coelobillardierine; 7,8-Didehydro	C9H9NO
		Coelobillardierine; Ketone	C9H9NO
		Cysteine, INN; (R)-form, S-Vinyl	C5H9NO2S
		Diethanolamine; N-Isopropyl	C7H17NO2
		Diethanolamine; N-Propyl	C7H17NO2
		3,4-Dihydro-1(2H)-isoquinolinone, 9CI	C9H9NO
		3,4-Dihydro-1(2H)-isoquinolinone, 9CI; NH-form	C9H9NO
		2,3-Dihydro-4(1H)-quinolinone, 9CI	C9H9NO
		3,4-Dihydroxybutanoic acid; (S)-form, Ethylamide	C6H13NO3
		2,4-Dihydroxy-3,3-dimethylbutanoic acid, 9CI; (R)-form, Amide	C6H13NO3
		2,4-Dihydroxy-3,3-dimethylbutanoic acid, 9CI; (S)-form, Amide	C6H13NO3
		2,4-Dihydroxy-3,3-dimethylbutanoic acid, 9CI; (±)-form, Amide	C6H13NO3
		3,4-Dihydroxy-2-(1-hydroxyethyl)pyrrolidine	C6H13NO3
		3,4-Dihydroxy-2-(1-hydroxyethyl)pyrrolidine; (1'R,2R,3R,4S)-form	C6H13NO3
		3,4-Dihydroxy-2-(hydroxymethyl)-5-methylpyrrolidine	C6H13NO3
		3,4-Dihydroxy-2-(hydroxymethyl)-5-methylpyrrolidine; (2R,3R,4R,5R)-form	C6H13NO3
		3,4-Dihydroxy-2-(hydroxymethyl)-5-methylpyrrolidine; (2S,3R,4S,5R)-form	C6H13NO3
		3,4-Dihydroxy-2-(hydroxymethyl)-5-methylpyrrolidine; (2S,3S,4S,5R)-form	C6H13NO3
		3,4-Dihydroxy-2-(hydroxymethyl)-5-methylpyrrolidine; (2R,3R,4S,5R)-form	C6H13NO3
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2R,3S,4R)-form	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2S,3R,4R)-form	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2S,3S,4S)-form	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2S,3S,4R)-form	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2S,3R,4S)-form	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2R,3R,4R)-form	C5H9NO4
		3,4-Dihydroxy-2-pyrrolidinecarboxylic acid; (2?,3?,4?)-form	C5H9NO4
		1-(2-Furanylmethyl)-1H-pyrrole, 9CI	C9H9NO

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
146.073 (continued)	**1000**	Glutamic acid	C5H9NO4
		Glutamic acid; (R)-form	C5H9NO4
		Glutamic acid; (S)-form	C5H9NO4
		Glutamic acid; (±)-form	C5H9NO4
		Glyoxylic acid, 8CI; Di-Et acetal, amide	C6H13NO3
		Glyoxylic acid, 8CI; Thiosemicarbazone	C3H5N3O2S
		2-Hydroxybenzoic acid, 9CI; Et ether, nitrile	C9H9NO
		3-Hydroxybenzoic acid, 9CI; Et ether, nitrile	C9H9NO
		4-Hydroxybenzoic acid, 8CI, 9CI; Et ether, nitrile	C9H9NO
		4-Hydroxy-3,5-dimethylbenzoic acid; Nitrile	C9H9NO
		N-Hydroxyglycine, 9CI; tert-Butyl ester	C6H13NO3
		N-Hydroxyleucine, 9CI	C6H13NO3
		N-Hydroxyleucine, 9CI; (R)-form	C6H13NO3
		N-Hydroxyleucine, 9CI; (S)-form	C6H13NO3
		N-Hydroxyleucine, 9CI; (±)-form	C6H13NO3
		2-Hydroxy-3-methyl-1H-indole	C9H9NO
		2-Hydroxy-3-methyl-1H-indole; (±)-form	C9H9NO
		2-Hydroxy-3-methyl-1H-indole; (?)-form	C9H9NO
		5-Hydroxy-3-methyl-1H-indole	C9H9NO
		2-(Hydroxymethyl)-3,4-piperidinediol, 9CI	C6H13NO3
		2-(Hydroxymethyl)-3,4-piperidinediol, 9CI; (2R,3R,4R)-form	C6H13NO3
		2-(Hydroxymethyl)-3,4-piperidinediol, 9CI; (2R,3R,4S)-form	C6H13NO3
		2-(Hydroxymethyl)-3,4-piperidinediol, 9CI; (2R,3S,4S)-form	C6H13NO3
		6-(Hydroxymethyl)-3,4-piperidinediol, 9CI	C6H13NO3
		6-(Hydroxymethyl)-3,4-piperidinediol, 9CI; (2S,4R,5R)-form	C6H13NO3
		6-(Hydroxymethyl)-3,4-piperidinediol, 9CI; (2S,4R,5S)-form	C6H13NO3
		6-(Hydroxymethyl)-3,4-piperidinediol, 9CI; (2S,4S,5R)-form	C6H13NO3
		8-Hydroxy-2,4,6-octatriynoic acid; Amide	C8H5NO2
		2-Hydroxyphenylacetic acid; Me ether, nitrile	C9H9NO
		3-Hydroxyphenylacetic acid; Me ether, nitrile	C9H9NO
		4-Hydroxyphenylacetic acid; Me ether, nitrile	C9H9NO
		2-Hydroxy-2-phenylacetonitrile; (±)-form, Me ether	C9H9NO
		2-Hydroxy-3-phenylpropanoic acid; (±)-form, Nitrile	C9H9NO
		3-(2-Hydroxyphenyl)propanoic acid; Nitrile	C9H9NO
		3-Hydroxy-3-phenylpropanoic acid; (S)-form, Nitrile	C9H9NO
		3-(4-Hydroxyphenyl)propanoic acid; Nitrile	C9H9NO
		1H-Indole-2,3-dione, 9CI	C8H5NO2
		1H-Indole-3-methanol	C9H9NO
		1H-Indole-7-methanol, 9CI	C9H9NO
		3H-Indol-3-one, 8CI, 9CI; 1-Oxide	C8H5NO2
		1-Isothiocyanato-3-(methylthio)propane, 9CI	C5H9NS2
		Malic acid, 8CI; (S)-form, 1-Amide, 4-Me ester	C5H9NO4
		Malic acid, 8CI; (R)-form, 4-Amide, 1-Me ester	C5H9NO4
		Malic acid, 8CI; (S)-form, 4-Amide, 1-Me ester	C5H9NO4
		Methyl [(acetylamino)oxy]acetate, 9CI	C5H9NO4

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
146.073 (continued)	**1000**	3,4-Methylenedioxybenzoic acid; Nitrile	C8H5NO2
		2-Methyl-3,4,5-piperidinetriol, 9CI	C6H13NO3
		2-Methyl-3,4,5-piperidinetriol, 9CI; (2R,3R,4R,5S)-form	C6H13NO3
		3-(Methylthio)-2-propenoic acid, 9CI; (Z)-form, Methylamide, S-oxide	C5H9NO2S
		3-(Methylthio)-2-propenoic acid, 9CI; (E)-form, Methylamide, S-oxide	C5H9NO2S
		3-Nitropropanoic acid, 9CI; Et ester	C5H9NO4
		3-Nitro-1-propanol, 8CI, 9CI; Ac	C5H9NO4
		Oxobutanedioic acid, 9CI; Oxo-form, Oxime	C4H5NO5
		2-Phenyl-2-propenal; Oxime	C9H9NO
		3-Phenyl-2-propenal, 9CI; (E)-form, E-Oxime	C9H9NO
		3-Phenyl-2-propenal, 9CI; (E)-form, Z-Oxime	C9H9NO
		3-Phenyl-2-propenoic acid, 9CI; (E)-form, Amide	C9H9NO
		Phthalimide, 8CI	C8H5NO2
		3,4,5-Piperidinetriol, 9CI; [(±)-(3a,4a,5ß)]-form, N-Me	C6H13NO3
		3,4,5-Piperidinetriol, 9CI; [3S-(3a,4a,5ß)]-form, N-Me	C6H13NO3
		2-Propenoic acid, 9CI; Anilide	C9H9NO
		Serine; (±)-form, N-Ac	C5H9NO4
		Serine; (S)-form, N-Ac	C5H9NO4
		Serine; (S)-form, O-Ac	C5H9NO4
		Serine; (±)-form, O-Ac	C5H9NO4
		5,6,7,8-Tetrahydro-4-methylquinoline	C10H13N
		3-Thiomorpholinecarboxylic acid, 9CI	C5H9NO2S
		3-Thiomorpholinecarboxylic acid, 9CI; (R)-form	C5H9NO2S
		3-Thiomorpholinecarboxylic acid, 9CI; (±)-form	C5H9NO2S
157.07	**1000**		
		Allantoin, USAN, BAN	C4H6N4O3
		Allantoin, USAN, BAN; (R)-form	C4H6N4O3
		Allantoin, USAN, BAN; (S)-form	C4H6N4O3
		Allantoin, USAN, BAN; (±)-form	C4H6N4O3
		2-Amino-4-methylenepentanedioic acid; (S)-form, ?-Amide	C6H10N2O3
		2-(Aminomethyl)-4-thiazolecarboxylic acid, 9CI	C5H6N2O2S
		2,3-Butanedione; Semicarbazone-oxime	C5H10N4O2
		Butanoic acid, 9CI; Anhydride	C8H14O3
		Cephalosporolide D	C8H14O3
		1,2-Cyclohexanediol, 9CI; (1S,2S)-form, Mono-Ac	C8H14O3
		2-Decene-4,6,8-triynoic acid	C10H6O2
		2-Decene-4,6,8-triynoic acid; (E)-form	C10H6O2
		2-Decene-4,6,8-triynoic acid; (Z)-form	C10H6O2
		1-Decene-4,6,8-triyn-3-one; 1?,2?-Epoxide	C10H6O2
		2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; (S)-form, Me ether	C7H10O4
		Dihydro-3-hydroxy-3-(1-hydroxyethyl)-4-methyl-2(3H)-furanone, 9CI; (1'S,3R,4R)-form, 1'-Ketone	C7H10O4
		4,5-Dihydroxy-3-(1-hydroxyethyl)-2-cyclopenten-1-one	C7H10O4
		4,5-Dihydroxy-3-(1-hydroxyethyl)-2-cyclopenten-1-one; (1'R,4R,5S)-form	C7H10O4
		4,5-Dihydroxy-4-(1-hydroxyethyl)-2-cyclopenten-1-one, 9CI	C7H10O4

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
157.07 (continued)	**1000**	3,5-Dihydroxy-2-hydroxymethyl-4H-pyran-4-one, 9CI	C6H6O5
		2,4-Dimethylquinazoline, 9CI	C10H10N2
		4,6-Dioxoheptanoic acid, 9CI	C7H10O4
		4,5-Dioxopentanoic acid; Et ester	C7H10O4
		2-Ethyl-3-methyl-2-butenedioic acid, 9CI	C7H10O4
		2-Ethyl-3-methyl-2-butenedioic acid, 9CI; (E)-form	C7H10O4
		2-Ethyl-3-methyl-2-butenedioic acid, 9CI; (Z)-form	C7H10O4
		6-Ethyl-1-methyl-2,7,8-trioxabicyclo[3.2.1]octane	C8H14O3
		5-Fluoro-2,4-dihydroxypyrimidine; 1,3-Di-Me	C6H7FN2O2
		5-Fluoro-2,4-dihydroxypyrimidine; 1-Et	C6H7FN2O2
		Heptanedioic acid, 9CI; Diamide	C7H14N2O2
		5-(2,4-Hexadiynylidene)-2(5H)-furanone	C10H6O2
		5-(2,4-Hexadiynylidene)-2(5H)-furanone; (E)-form	C10H6O2
		5-(2,4-Hexadiynylidene)-2(5H)-furanone; (Z)-form	C10H6O2
		Hexahydro-3a,6(4H)-benzofurandiol, 9CI	C8H14O3
		Hexahydro-3a,6(4H)-benzofurandiol, 9CI; (3aR*,6R*,7aS*)-form	C8H14O3
		Hexahydro-3a,6(4H)-benzofurandiol, 9CI; (3aR*,6S*,7aS*)-form	C8H14O3
		3-Hydroxy-2-butanone, 8CI, 9CI; (±)-form, Butanoyl	C8H14O3
		10-Hydroxy-8-decene-2,4,6-triynal	C10H6O2
		10-Hydroxy-8-decene-2,4,6-triynal; (E)-form	C10H6O2
		3-(1-Hydroxyethyl)-2,5-piperazinedione, 9CI	C6H10N2O3
		3-(1-Hydroxyethyl)-2,5-piperazinedione, 9CI; (1'R,3S)-form	C6H10N2O3
		3-(1-Hydroxyethyl)-2,5-piperazinedione, 9CI; (1'?,3?)-form	C6H10N2O3
		1-Hydroxy-3-hexanone, 9CI; Ac	C8H14O3
		6-Hydroxy-3-hexanone, 9CI; Ac	C8H14O3
		4-Hydroxy-4-(2-hydroxyethyl)cyclohexanone, 9CI	C8H14O3
		4-Hydroxy-3-methyl-2-butenoic acid, 9CI; (E)-form, Ac	C7H10O4
		4-Hydroxy-2-methylenebutanoic acid; Ac	C7H10O4
		5-Hydroxy-4-methyl-2-heptenoic acid	C8H14O3
		5-Hydroxy-4-methyl-2-heptenoic acid; (2E,4S,5R)-form	C8H14O3
		5-Hydroxy-4-methyl-2-heptenoic acid; (2E,4S,5S)-form	C8H14O3
		3-Hydroxy-5-octenoic acid	C8H14O3
		3-Hydroxy-5-octenoic acid; (3R,5Z)-form	C8H14O3
		3-Hydroxy-5-octenoic acid; (3S,5E)-form	C8H14O3
		3-Hydroxy-5-octenoic acid; (±)-(E)-form	C8H14O3
		6-Hydroxy-7-octenoic acid	C8H14O3
		6-Hydroxy-7-octenoic acid; (R)-form	C8H14O3
		3-Hydroxypropanal, 9CI; Tetrahydropyranyl ether	C8H14O3
		Ibotenic acid	C5H6N2O4
		Ibotenic acid; (±)-form	C5H6N2O4
		Imidazole, 9CI, 8CI; N-Benzyl	C10H10N2
		1H-Indole-3-carboxylic acid; N-Hydroxy, nitrile	C9H6N2O
		Isoquinoline, 9CI, 8CI; N-Et	C11H12N
		2-Methyl-2-butenedioic acid, 9CI; (E)-form, Di-Me ester	C7H10O4
		2-Methyl-2-butenedioic acid, 9CI; (Z)-form, Di-Me ester	C7H10O4
		2-Methyl-2-butene-1,4-diol; (E)-form, 4-Me ether, 1-Ac	C8H14O3
		2-Methyl-2-butene-1,4-diol; (Z)-form, 4-Me ether, 1-Ac	C8H14O3

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
157.07 (continued)	**1000**	Methylenebutanedioic acid, 9CI; Di-Me ester	C7H10O4
		Methylenebutanedioic acid, 9CI; 4-Mono-Et ester	C7H10O4
		2-Methylene-4-oxopentanedioic acid	C6H6O5
		5-Methyl-2,3-hexanedione, 9CI; Dioxime	C7H14N2O2
		2-Methylpropanoic acid, 9CI; Anhydride	C8H14O3
		2-Methyl-2-propenoic acid, 9CI; 2-Ethoxyethyl ester	C8H14O3
		1-Methyl-4(1H)-quinolinone, 9CI; Imine	C10H10N2
		Muscazone	C5H6N2O4
		Muscazone; (±)-form	C5H6N2O4
		2-Nonene-4,6,8-triynoic acid, 9CI; (E)-form, Me ester	C10H6O2
		2-Oxohexanoic acid, 9CI; Et ester	C8H14O3
		2-Oxo-4-methylenepentanedioic acid	C6H6O5
		2-Oxooctanoic acid, 9CI	C8H14O3
		4-Oxopentanoic acid, 9CI; Isopropyl ester	C8H14O3
		4-Oxopentanoic acid, 9CI; Propyl ester	C8H14O3
		3-Oxopropanoic acid, 9CI; (E)-Enol-form, Et ester, Ac	C7H10O4
		5-Phenyl-2,4-pentadienal, 9CI	C11H10O
		5-Phenyl-2,4-pentadienal, 9CI; (E,E)-form	C11H10O
		5-Phenyl-2,4-pentadienal, 9CI; (2Z,4E)-form	C11H10O
		1,2,3-Propanetricarboxylic acid, 9CI, 8CI; 1,2-Anhydride	C6H6O5
		3-(2-Pyrrolyl)pyridine; N-Me	C10H10N2
		Pyruvic acid, 8CI; 3-Methylbutyl ester	C8H14O3
		Rengyoxide	C8H14O3
		Terrestric acid	C4H6N4O3
		Tetrahydro-2,5-furandicarboxylic acid; (2RS,5SR)-form, Diamide	C6H10N2O3
		Tetrahydro-2-furanmethanol, 9CI; (±)-form, Propanoyl	C8H14O3
		1,4,5,6-Tetrahydro-5-hydroxy-2-methyl-4(6)-pyrimidinecarboxylic acid	C6H10N2O3
		Tetrahydro-6-methyl-2H-pyran-2-acetic acid	C8H14O3
		Tetrahydro-6-methyl-2H-pyran-2-acetic acid; (2S,6S)-form	C8H14O3
		Tetrahydro-6-methyl-2H-pyran-2-acetic acid; (2RS,6RS)-form	C8H14O3
		Tetrahydro-6-methyl-2H-pyran-2-acetic acid; (2RS,6SR)-form	C8H14O3
		2,3,4-Trihydroxybutanoic acid, 9CI; (2R,3R)-form, 1,4-Lactone, 2,3-isopropylidene	C7H10O4
		2,3,4-Trihydroxybutanoic acid, 9CI; (2S,3S)-form, Lactone, 2,3-O-isopropylidene	C7H10O4
		4,5,6-Trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-one; (4R*,5S*,6R*)-form, 1'-Deoxy	C7H10O4
		2,5,6-Trihydroxy-3-methyl-2-cyclohexen-1-one	C7H10O4
		2,5,6-Trihydroxy-3-methyl-2-cyclohexen-1-one; (5R,6R)-form	C7H10O4
		3,4,5-Trihydroxy-5-methyl-2-cyclohexen-1-one	C7H10O4
		3,4,5-Trihydroxy-5-methyl-2-cyclohexen-1-one; (4R*,5S*)-form	C7H10O4
		3,4,5-Trihydroxy-5-methyl-2-cyclohexen-1-one; (4R,5R)-form	C7H10O4

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
		3,4,6-Trihydroxy-2-methyl-2-cyclohexen-1-one	C7H10O4
		3,4,6-Trihydroxy-2-methyl-2-cyclohexen-1-one; (4R,6R)-form	C7H10O4
		4,5,6-Trihydroxy-2-methyl-2-cyclohexen-1-one	C7H10O4
		4,5,6-Trihydroxy-2-methyl-2-cyclohexen-1-one; (4R,5R,6S)-form	C7H10O4
		4,5,6-Trihydroxy-2-methyl-2-cyclohexen-1-one; (4R,5R,6R)-form	C7H10O4
		Valine; (±)-form, N-Ac, amide	C7H14N2O2
		Zymonic acid	C6H6O5
337.121	**50**		
		Acremonin A; (+)-form, 6-O-ß-D-Glucopyranoside	C17H22O7
		Alangimaridine; (R)-form, 14?-Hydroxy, 13,13a-didehydro, 14,15-dihydro	C19H18N2O4
		Alangimaridine; (R)-form, 14?-Hydroxy, 13,13a-didehydro, 14,15-dihydro, O-de-Me, O2-Me	C19H18N2O4
		Alpinumisoflavone; 3,4-Dihydro	C20H18O5
		3-Amino-3-pyrrolidinecarboxylic acid, 9CI; (S)-form, N,N'-Dibenzoyl	C19H18N2O4
		Anislactone A; 7-Epimer, 7-Ac	C17H22O7
		Antibiotic C 15462; Antibiotic C 15462V	C20H18O5
		Antibiotic MK 3018	C20H18O5
		Antibiotic Sch 58450	C20H18O5
		Apiocarpin	C20H18O5
		Aplysinopsin; Di-Ac	C18H18N4O3
		Bakuchalcone; 2-Hydroxy, 3"-deoxy, 3",4"-didehydro	C20H18O5
		17(15?16),18(4?3)-Bisabeo-12,16-epoxy-11-hydroxy-3,5,8,11,13-abietapentaen-7-one; 1-Oxo, 14-hydroxy, 15,16-didehydro	C20H18O5
		Buceracidin B	C20H18O5
		Capitellataquinone B; 3',8-Dideoxy	C20H18O5
		Carpachromene; 2,3-Dihydro	C20H18O5
		Carpachromene; 3",4"-Dihydro	C20H18O5
		Carrabiose; a-Pyranose-form, Me glycoside	C13H22O10
		Carrabiose; ß-Pyranose-form, Me glycoside	C13H22O10
		Caseadinium(1+)	C20H20NO4
		Citflavanone	C20H18O5
		Citflavanone; (S)-form	C20H18O5
		Citflavanone; (?)-form	C20H18O5
		Citflavanone; (±)-form	C20H18O5
		Cladosporol A; Deepoxy	C20H18O5
		Corylin; 3',4'-Dihydro, 3'-hydroxy	C20H18O5
		Curcumin; Demethoxy	C20H18O5
		Cyclocalopin A; 7-Ac	C17H22O7
		Cyclokievitone; 5-Deoxy	C20H18O5
		Daleformis	C20H18O5
		Decaspirone F	C20H18O5
		Decaspirone F; 6,7-Dihydro, 4-ketone	C20H18O5
		5-Deoxy-3-C-hydroxymethyllyxose, 9CI; a-L-Furanose-form, Benzyl glycoside, 2,3'-di-O-Ac	C17H22O7

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
337.121	**50**	2,4-Diamino-2,4,6-trideoxy-3-(a-D-galactopyranuronosyl)-D-galactose	C12H22N2O9
(continued)		2,4-Diamino-2,4,6-trideoxy-3-(a-D-galactopyranuronosyl)-D-galactose; a-Pyranose-form	C12H22N2O9
		2,6-Diamino-2,3,6-trideoxy-ribo-hexose; a-D-Pyranose-form, Me glycoside, N,N'-di-Ac, 4-mesyl	C12H22N2O7S
		1,2:3,4-Diepoxy-8,10-dihydroxy-12,6-guaianolide; (1ß,2ß,3ß,4ß,6a,8a,10a,11a)-form, 8-Ac	C17H22O7
		1,2:3,4-Diepoxy-8,10-dihydroxy-11(13)-guaien-12,6-olide; (1ß,2ß,3ß,4ß,5a,6a,8a,10a)-form, 11ß,13-Dihydro, 8-Ac	C17H22O7
		7,8-Dihydro-5,7-dihydroxy-2,2-dimethyl-8-phenyl-2H,6H-benzo[1,2-b:5,4-b']dipyran-6-one	C20H18O5
		7,8-Dihydro-5,7-dihydroxy-2,2-dimethyl-8-phenyl-2H,6H-benzo[1,2-b:5,4-b']dipyran-6-one; (2R*,3R*)-form	C20H18O5
		3,4-Dihydro-2-methyl-2H-pyran-3,4-diol; (2S,3R,4S)-form, 3,4-Dibenzoyl	C20H18O5
		5,7-Dihydroxy-8-(3-hydroxy-3-methylbutyl)-2-methyl-4H-1-benzopyran-4-one; 1',2'-Dihydroxy, 5,7-di-Me ether	C17H22O7
		4,7-Dihydroxy-5-[2-(4-hydroxyphenyl)ethenyl]-3-methyl-2H-1-benzopyran-2-one; (E)-form, 4,7-Di-Me ether	C20H18O5
		5,7-Dihydroxy-8-(3-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one	C20H18O5
		2,10-Dihydroxy-3-oxo-1,11(13)-eremophiladien-12-oic acid; 10ß-form, 10-Hydroperoxide, 2-Ac	C17H22O7
		2-(3,4-Dihydroxyphenyl)-2,3-dihydro-3-methyl-7-(2-propenyl)-4,5-benzofurandiol; (7R*,8R*)-form, 3',4':4,5-Bis(methylene) ether	C20H18O5
		5,7-Dihydroxy-8-prenylflavanone; (S)-form, 4"-Oxo	C20H18O5
		2-(2,4-Dihydroxy-5-prenylphenyl)-5,6-dihydroxybenzofuran; 5,6-Methylene ether	C20H18O5
		4-[(Di-1H-indol-3-yl)methyl]phenol, 9CI	C23H18N2O
		2,2-Dimethyl-2H-1-benzopyran-7-ol, 9CI; O-ß-D-Glucopyranoside	C17H22O7
		8-(1,1-Dimethyl-2-propenyl)-3,5,7-trihydroxyflavone	C20H18O5
		6-(1,1-Dimethyl-2-propenyl)-4',5,7-trihydroxyisoflavone	C20H18O5
		8-(1,1-Dimethyl-2-propenyl)-4',5,7-trihydroxyisoflavone	C20H18O5
		Dugesin A	C20H18O5
		15,16-Epoxy-1,3,7,13(16),14-clerodapentaene-17,12:18,19-diolide	C20H18O5
		4,5-Epoxy-8,13-dihydroxy-1-oxo-7(11)-germacren-12,6-olide; (4a,5ß,6a,8a,10a)-form, 13-Ac	C17H22O7
		7,8-Epoxy-9,17-dihydroxy-13,14,15,16-tetranor-12,17:19,6-labdanediolide; (6ß,7a,8a,9a,17a)-form, 17-Me ether	C17H22O7
		1,4-Epoxy-1,8,13-trihydroxy-5,7(11)-germacradien-12,6-olide; (1a,4ß,5E,8a,10ßH)-form, 13-Ac	C17H22O7
		Eschscholtzine; (-)-form, N-Me	C20H20NO4

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
337.121 (continued)	**50**	Ezomontanin; 11ß,13-Dihydro	C17H22O7
		Glyasperin F; 5-Deoxy	C20H18O5
		Glyceollin I	C20H18O5
		Glyceollin II	C20H18O5
		Glyceollin III	C20H18O5
		Glyceollin III; 13-Epimer	C20H18O5
		Glyinflanin B	C20H18O5
		Hatomarubigin C	C20H18O5
		2-Hydroxy-1,5,8,10(20),13-icetexapentaene-3,7,11,12-tetrone	C20H18O5
		5-Hydroxy-7-methoxy-8-(3-oxo-1-butenyl)flavanone	C20H18O5
		5-Hydroxy-7-methoxy-8-(3-oxo-1-butenyl)flavanone; (S,E)-form	C20H18O5
		1-Hydroxy-2-(4-methyl-3-pentenyl)anthraquinone; 3,4-Dihydroxy	C20H18O5
		4'-Hydroxyongokein; (1'RS,2S,4'SR)-form, 4'-Ketone, di-Me acetal	C17H22O7
		6a-Hydroxyphaseollin	C20H18O5
		1a-Hydroxyphaseollone	C20H18O5
		6-Hydroxy-5(10)-pinguisen-11,6-olide-12,15-dioic acid; (6aOH)-form, Di-Me ester	C17H22O7
		Iduronic acid; ß-L-Furanose-form, 1,2-Isopropylidene, 3-benzyl, Me ester	C17H22O7
		Isobavachromene; 2"-Hydroxy	C20H18O5
		Isobavachromene; 3"-Hydroxy	C20H18O5
		Licoisoflavone B; 2,3-Dihydro, 5-deoxy	C20H18O5
		Mallotus A	C20H18O5
		Moracin G; 4'-Methoxy	C20H18O5
		Murragleinin	C17H22O7
		Neoraufurane	C20H18O5
		Otobain; (-)-form, 8-Epimer, 7-oxo	C20H18O5
		Otobain; (-)-form, 7-Oxo	C20H18O5
		Ovigerine; (±)-form, N,N-Di-Me	C20H20NO4
		Oxazinine 1; O-De(cyanoethyl)	C19H18N2O4
		Palmarumycin CP3; 6,7-Dihydro, 5ß-alcohol	C20H18O5
		Palmatine; O10-De-Me	C20H20NO4
		Palmatine; O2-De-Me	C20H20NO4
		Palmatine; O3-De-Me	C20H20NO4
		Palmatine; O9-De-Me	C20H20NO4
		Pinnatal	C20H18O5
		Pinnatal; 9-Deoxy, 8-hydroxy	C20H18O5
		Prehalenaquinone; Hydroquinone	C20H18O5
		Pseudopalmatine; O2-De-Me	C20H20NO4
		Pseudopalmatine; O3-De-Me	C20H20NO4
		Psorolactone B	C20H18O5
		Reframidine; (-)-form, N-Me	C20H20NO4
		Reframidine; (±)-form, N-Me	C20H20NO4
		Rhyacophilin; 7,8-Didehydro	C20H18O5
		Rubiginone A1	C20H18O5
		3,7,8,15-Scirpenetetrol; 8-Ketone, 15-Ac	C17H22O7
		Sterekunthal A	C20H18O5

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
337.121 (continued)	**50**	Stylopine, 9CI; (S)-form, N-Me (cis-)	C20H20NO4
		Stylopine, 9CI; (S)-form, N-Me (trans-)	C20H20NO4
		Tanshinonic acid; Me ester	C20H18O5
		Terphenyllin	C20H18O5
		3',4,4',5-Tetrahydroxy-2,7'-cyclolignan-7-one; (7'R,8R,8'S)-form, 3',4':4,5-Bis(methylene) ether	C20H18O5
		3',4,4',5-Tetrahydroxy-2,7'-cyclolignan-7-one; (7'R,8S,8'R)-form, 3',4':4,5-Bis(methylene) ether	C20H18O5
		3',4,4',5-Tetrahydroxy-2,7'-cyclolignan-7-one; (7'R,8S,8'S)-form, 3',4':4,5-Bis(methylene) ether	C20H18O5
		1,3,4,8-Tetrahydroxy-9,11(13)-guaiadien-12,6-olide; (1a,3a,4ß,5a,6a,8a)-form, 8-Ac	C17H22O7
		2,8,9,10-Tetrahydroxy-3,11(13)-guaiadien-12,6-olide; (1a,2ß,5a,6a,8a,9a,10a)-form, 9-Ac	C17H22O7
		3,4,8,10-Tetrahydroxy-1,11(13)-guaiadien-12,6-olide; (3a,4a,5a,6a,8a,10ß)-form, 8-Ac	C17H22O7
		3,4,8,10-Tetrahydroxy-1,11(13)-guaiadien-12,6-olide; (3ß,4a,5a,6a,8a,10ß)-form, 8-Ac	C17H22O7
		3,8,9,10-Tetrahydroxy-4,11(13)-guaiadien-12,6-olide; (1a,3a,6a,8a,9a,10a)-form, 8-Ac	C17H22O7
		Tetrangomycin; (?)-form, 1?-Alcohol, O8-Me	C20H18O5
		Tetrangomycin; (?)-form, Stereoisomer (?), 1?-alcohol, O8-Me	C20H18O5
		Teuvincenone E; 15,16-Didehydro	C20H18O5
		1,3,8-Trihydroxy-4,10(14),11(13)-germacratrien-12,6-olide; (1ß,3ß,4E,6a,8ß)-form, 1-Hydroperoxide, 3-Ac	C17H22O7
		1,8,15-Trihydroxy-4,10(14),11(13)-germacratrien-12,6-olide; (1ß,4E,6a,8a)-form, 1-Hydroperoxide, 15-Ac	C17H22O7
		4,9,10-Trihydroxy-2,11(13)-guaiadien-12,6-olide; (4a,6a,9a,10a)-form, 4-Hydroperoxide, 9-Ac	C17H22O7
		1,3,8-Trihydroxy-6-methylanthraquinone, 8CI; 3-O-(3-Methyl-2-butenyl)	C20H18O5
		1,3,8-Trihydroxy-6-methyl-2-(3-methyl-2-butenyl)anthraquinone	C20H18O5
		8,9,13-Trihydroxy-1-oxo-4,7(11)-germacradien-12,6-olide; (4E,6a,8a,9ß,10ß)-form, 13-Ac	C17H22O7
		9,11,13-Trihydroxy-4-oxo-2-pseudoguaien-12,8-olide; (1a,5ß,8a,9ß,10a,11ß)-form, 9-Ac	C17H22O7
		3',4',6-Trihydroxy-5-prenylaurone	C20H18O5
		3',4',6-Trihydroxy-7-prenylaurone	C20H18O5
		3',4',7-Trihydroxy-6-prenylflavone	C20H18O5
		3',4',7-Trihydroxy-8-prenylflavone	C20H18O5
		3,5,7-Trihydroxy-6-prenylflavone	C20H18O5
		4',5,7-Trihydroxy-3'-prenylflavone	C20H18O5
		4',5,7-Trihydroxy-6-prenylflavone	C20H18O5
		4',5,7-Trihydroxy-8-prenylflavone	C20H18O5
		2',4',7-Trihydroxy-3'-prenylisoflavone	C20H18O5
		3',4',7-Trihydroxy-5'-prenylisoflavone	C20H18O5
		3',4',7-Trihydroxy-8-prenylisoflavone	C20H18O5

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
337.121 (continued)	**50**	3',4',7-Trihydroxy-8-prenylisoflavone	C20H18O5
		4',5,7-Trihydroxy-3'-prenylisoflavone	C20H18O5
		4',5,7-Trihydroxy-6-prenylisoflavone	C20H18O5
		4',5,7-Trihydroxy-8-prenylisoflavone	C20H18O5
		3,8,9-Trihydroxy-7-prenylpterocarpene	C20H18O5
		3,7,15-Trihydroxy-8-scirpenone; (3a,7a)-form, 3-Ac	C17H22O7
		Tryptophan, 9CI, USAN, 8CI; (R)-form, Na-Benzyloxycarbonyl	C19H18N2O4
		Tuberosin	C20H18O5
		Tuberosin; (+)-form	C20H18O5
		Tuberosin; (-)-form	C20H18O5
		Vismiaguianin A	C20H18O5
		Vitrofolal B; 3-Deoxy	C20H18O5
315.126	**500**		
		2-Acetyl-1,3,6,8-tetrahydroxyanthraquinone; 1'R-Alcohol	C16H12O7
		2-Amino-3-(3-bromo-4-hydroxyphenyl)propanoic acid; (S)-form, 4-Me ether, N,N,N-tri-Me, betaine	C13H18BrNO3
		1,5-Anhydroglucitol, 9CI, 8CI; D-form, 2-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		1,5-Anhydroglucitol, 9CI, 8CI; D-form, 6-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		Boxazomycin A	C14H12N4O5
		6-Bromo-1,2,3,4-tetrahydro-7,8-dihydroxy-3-isoquinolinecarboxylic acid; (S)-form, 7-Me ether, Me ester	C12H14BrNO4
		8-Bromo-1,2,3,4-tetrahydro-6,7-dihydroxy-3-isoquinolinecarboxylic acid; (S)-form, 7-Me ether, Me ester	C12H14BrNO4
		Coccineone D	C16H12O7
		Crombeone	C16H12O7
		1,1-Dibromo-2-heptanol; (±)-form, Ac	C9H16Br2O2
		2',5'-Dihydroxyacetophenone, 8CI; Bis(2,2,2-trifluoroethyl) ether	C12H10F6O3
		2,3-Dihydroxybenzoic acid, 9CI; 3-O-ß-D-Glucopyranoside	C13H16O9
		2,5-Dihydroxybenzoic acid, 9CI; 2-O-ß-D-Glucopyranoside	C13H16O9
		2,5-Dihydroxybenzoic acid, 9CI; 5-O-ß-D-Glucopyranoside	C13H16O9
		3,4-Dihydroxybenzoic acid; 3-O-ß-D-Glucopyranoside	C13H16O9
		3,4-Dihydroxybenzoic acid; 4-O-ß-D-Glucopyranoside	C13H16O9
		3-(3,4-Dihydroxybenzylidene)-5,6,7-trihydroxy-4-chromanone	C16H12O7
		3-(3,4-Dihydroxybenzylidene)-5,6,7-trihydroxy-4-chromanone; (Z)-form	C16H12O7
		3-(3,4-Dihydroxybenzylidene)-5,7,8-trihydroxy-4-chromanone	C16H12O7

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
315.126	**500**	3-(3,4-Dihydroxybenzylidene)-5,7,8-trihydroxy-4-chromanone; (Z)-form	C16H12O7
(continued)		1,9-Dihydroxy-3-hydroxymethyl-10-methoxydibenz[b,e]oxepin-6,11-dione	C16H12O7
		2,8-Dihydroxy-6-hydroxymethylxanthone-1-carboxylic acid; Me ester	C16H12O7
		2,8-Dihydroxy-6-hydroxymethylxanthone-1-carboxylic acid; 8-Me ether	C16H12O7
		2-(3,4-Dihydroxyphenyl)-4,6-dihydroxy-3-benzofurancarboxylic acid; Me ester	C16H12O7
		1-(2,4-Dihydroxyphenyl)-2-(2,4,5-trihydroxyphenyl)ethanedione; 4,5-Methylene, 4'-Me ether	C16H12O7
		Ellagic acid, INN; 3-Me ether	C15H8O8
		1,2-Epoxy-8-hydroxy-6-(hydroxymethyl)-1-methoxycarbonylxanthone	C16H12O7
		Fusidienol	C16H12O7
		Laughine	C11H18BrN5O
		Lindbladiapyrone	C16H12O7
		Orotidine; 3N-Me, Me ester	C12H16N2O8
		Pannaric acid	C16H12O7
		1,2,3,5,8-Pentahydroxyanthraquinone; 1,3-Di-Me ether	C16H12O7
		1,2,3,7,8-Pentahydroxyanthraquinone; 2,3-Di-Me ether	C16H12O7
		3',4,4',5',6-Pentahydroxyaurone; 4-Me ether	C16H12O7
		1,2,3,8,9-Pentahydroxycoumestan	C15H8O8
		2',3',5,6,7-Pentahydroxyflavanone; (S)-form, 6,7-Methylene ether	C16H12O7
		2',3,4',5,7-Pentahydroxyflavone; 2'-Me ether	C16H12O7
		2',3,4',5,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		2',3,4',5,7-Pentahydroxyflavone; 5-Me ether	C16H12O7
		2',3',4',6,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		2',3,5,7,8-Pentahydroxyflavone; 7-Me ether	C16H12O7
		2',4',5,6,7-Pentahydroxyflavone; 6-Me ether	C16H12O7
		2',5,6',7,8-Pentahydroxyflavone; 8-Me ether	C16H12O7
		3,3',4',6,7-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,3',4',6,7-Pentahydroxyflavone; 7-Me ether	C16H12O7
		3,3',4',7,8-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,3',4',7,8-Pentahydroxyflavone; 3'-Me ether	C16H12O7
		3,3',4',7,8-Pentahydroxyflavone; 8-Me ether	C16H12O7
		3',4',5,5',7-Pentahydroxyflavone; 3'-Me ether	C16H12O7
		3',4',5,5',7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3,4',5,6,7-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,4',5,6,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyflavone; 5-Me ether	C16H12O7
		3,4',5,6,7-Pentahydroxyflavone; 7-Me ether	C16H12O7
		3,4',5,7,8-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,4',5,7,8-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3,4',5,7,8-Pentahydroxyflavone; 7-Me ether	C16H12O7
		3',4',5,7,8-Pentahydroxyflavone; 8-Me ether	C16H12O7
		3,5,6,7,8-Pentahydroxyflavone; 3-Me ether	C16H12O7

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
315.126 (continued)	**500**	4',5,6,7,8-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		4',5,6,7,8-Pentahydroxyflavone; 6-Me ether	C16H12O7
		2',4',5,5',7-Pentahydroxyisoflavone; 5'-Me ether	C16H12O7
		3',4',5,5',7-Pentahydroxyisoflavone; 4'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyisoflavone; 3'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyisoflavone; 6-Me ether	C16H12O7
		3',4',5,7,8-Pentahydroxyisoflavone; 8-Me ether	C16H12O7
		4',5,6,7,8-Pentahydroxyisoflavone; 8-Me ether	C16H12O7
		1,2,3,4,5-Pentahydroxy-7-methylanthraquinone; 2-Me ether	C16H12O7
		1,2,3,7,8-Pentahydroxy-6-methylanthraquinone; 1-Me ether	C16H12O7
		1,2,3,7,8-Pentahydroxy-6-methylanthraquinone; 2-Me ether	C16H12O7
		1,3,4,5,7-Pentahydroxy-2-methylanthraquinone; 7-Me ether	C16H12O7
		1,3,5,6,8-Pentahydroxy-2-methylanthraquinone; 6-Me ether	C16H12O7
		1,3,5,7,8-Pentahydroxy-2-methylanthraquinone; 7-Me ether	C16H12O7
		3',4,4',5',6-Pentahydroxy-5-methylaurone	C16H12O7
		3,3',4',5,7-Pentahydroxy-6-methylflavone	C16H12O7
		3,3',4',5,7-Pentahydroxy-8-methylflavone	C16H12O7
		3,4',5,6,7-Pentahydroxy-8-methylflavone	C16H12O7
		3',4',5,6,7-Pentahydroxy-8-methylflavone	C16H12O7
		3,4',5,7,8-Pentahydroxy-6-methylflavone	C16H12O7
		3',4',5,5',7-Pentahydroxy-6-methylisoflavone	C16H12O7
		3,6a,7,8,9-Pentahydroxypterocarpan; 8,9-Methylene ether	C16H12O7
		proto-Quercitol; L-form, 1-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		scyllo-Quercitol, 9CI, 8CI; 2-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		1,3,5,6-Tetrahydroxy-2-hydroxymethylanthraquinone; 6-Me ether	C16H12O7
		3',4',5,7-Tetrahydroxy-3-hydroxymethylflavone	C16H12O7
		3,3',4',5-Tetrahydroxy-7-methoxyflavone	C16H12O7
		3,3',4',7-Tetrahydroxy-5-methoxyflavone	C16H12O7
		3,3',5,7-Tetrahydroxy-4'-methoxyflavone	C16H12O7
		3',4',5,6-Tetrahydroxy-7-methoxyflavone	C16H12O7
		3,4',5,7-Tetrahydroxy-3'-methoxyflavone	C16H12O7
		3',4',5,7-Tetrahydroxy-3-methoxyflavone	C16H12O7
		3,4',5,7-Tetrahydroxy-6-methoxyflavone	C16H12O7
		3',4',5,7-Tetrahydroxy-6-methoxyflavone	C16H12O7
		3,4',5,7-Tetrahydroxy-8-methoxyflavone	C16H12O7
		3,4',6,7-Tetrahydroxy-5-methoxyflavone	C16H12O7
		3',5,7,8-Tetrahydroxy-4'-methoxyflavone	C16H12O7
		4',5,6,7-Tetrahydroxy-3'-methoxyflavone	C16H12O7
		4',5,7,8-Tetrahydroxy-3'-methoxyflavone	C16H12O7
		4,9,11,12a-Tetrahydroxyrotenoid	C16H12O7
		3,4,5-Trihydroxybenzaldehyde, 9CI; 4-O-ß-D-Glucopyranoside	C13H16O9

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
315.126	**500**	3,4,5-Trihydroxybenzoic acid; a-L-Rhamnopyranosyl ester	C13H16O9
(continued)		2,5,7-Trihydroxy-4H-1-benzopyran-4-one; 2-O-(3-Hydroxy-4-methoxyphenyl)	C16H12O7
		5,6,7-Trihydroxy-2-(4-hydroxyphenoxy)-4H-1-benzopyran-4-one; 6-Me ether	C16H12O7
		5,7,10-Trihydroxy-3-methyl-1H-naphtho[2,3-c]pyran-1,6,9-trione; 5,7-Di-Me ether	C16H12O7
		2,5,8-Trihydroxy-6-methylxanthone-1-carboxylic acid; 8-Me ether	C16H12O7
		2,3,7-Trihydroxy-1,6-phenazinedicarboxylic acid, 9Cl	C14H8N2O7
		Uridine-5-acetic acid; Me ester	C12H16N2O8
		Verrucarin G	C15H12N2O6
473.201	**100**		
		Acremolactone A	C26H34O8
		Agrimophol	C26H34O8
		Amoorastatin; O3-De-Ac	C26H34O8
		Austalide B	C26H34O8
		Clausenolide; 1-Me ether	C26H34O8
		Cryptochlorophaeic acid; O-De-Me, 4,4'-di-Me ether	C26H34O8
		Cryptochlorophaeic acid; 4-Me ether	C26H34O8
		Cryptochlorophaeic acid; 4'-Me ether	C26H34O8
		14,15:19,29-Diepoxy-1,3,7,29-tetrahydroxy-11-meliacanone	C26H34O8
		14,15:19,29-Diepoxy-1,3,7,29-tetrahydroxy-11-meliacanone; (1a,3a,7a,14ß,15ß,29R)-form	C26H34O8
		7,8:17,18-Diepoxy-1(19),6(20),10(17),13-xenicatetraene-11,12,18-triol; Tri-Ac	C26H34O8
		4,8-Dihydroxy-2,10(14),11(13)-guaiatrien-12,6-olide; (1a,4a,5a,6a,8ß)-form, 8-O-[4-Hydroxy-2-(2-hydroxymethyl-2E-butenoyloxy)methyl-2E-butenoyl]	C25H30O9
		Epiphorellic acid 1	C26H34O8
		15,16-Epoxy-1,2-dihydroxy-3,13(16),14-clerodatrien-17,12-olid-18-oic acid; (ent-1ß,2ß,12ßH)-form, Di-Ac, Me ester	C25H30O9
		10,14-Epoxy-2,8-dihydroxy-3,11(13)-guaiadien-12,6-olide; (1a,2ß,5a,6a,8ß,10a)-form, 8-[2-(2-Hydroxymethyl-2E-butenoyloxymethyl)-2E-butenoyl]	C25H30O9
		19,29-Epoxy-1,3,7,12,29-pentahydroxy-14-meliacen-11-one	C26H34O8
		19,29-Epoxy-1,3,7,12,29-pentahydroxy-14-meliacen-11-one; (1a,3a,7a,12a,29R)-form	C26H34O8
		14,15-Epoxy-3,5,16,19-tetrahydroxybufa-20,22-dienolide; (3ß,5ß,14ß,15ß,16ß)-form, 16-Ac	C26H34O8
		19,29-Epoxy-1,3,7,29-tetrahydroxymeliacane-11,15-dione	C26H34O8
		19,29-Epoxy-1,3,7,29-tetrahydroxymeliacane-11,15-dione; (1a,3a,7a,29R)-form	C26H34O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
473.201	**100**	8,17-Epoxy-2,9,12-trihydroxy-5,13-briaradien-18,7-olide; (2ß,5Z,7a,8a,9ß,12a,17a)-form, 12-Ketone, 2-butanoyl, 9-Ac	C26H34O8
(continued)		3',4,4',5,5',9,9'-Heptahydroxy-2,7'-cyclolignan; (7'S,8R,8'R)-form, 3',5,5'-Tri-Me ether, 9,9'-di-Ac	C25H30O9
		3,3',4,4',5,9,9'-Heptahydroxylignan; (8R,8'R)-form, 3',4'-Methylene, 3,5-di-Me ether, 9,9'-di-Ac	C25H30O9
		1,3,5,11,14,19-Hexahydroxybufa-20,22-dienolide; (1ß,3ß,5ß,11a,14ß)-form, 1,3,5-Orthoacetate	C26H34O8
		3',4,4',5,9,9'-Hexahydroxy-2,7'-cyclolignan; (7'S,8R,8'R)-form, 3',5-Di-Me ether, 9-O-(5-hydroxy-4-oxopentanoyl)	C25H30O9
		3',4,4',5,9,9'-Hexahydroxy-2,7'-cyclolignan; (7'S,8R,8'R)-form, 3',5-Di-Me ether, 9'-O-(5-hydroxy-4-oxopentanoyl)	C25H30O9
		3,3',4,4',9,9'-Hexahydroxylignan; (8R,8'R)-form, 3,3',4,4'-Tetra-Me ether, 9,9'-di-Ac	C26H34O8
		8-Hydroxy-1(10),3,11(13)-guaiatrien-12,6-olide; (5a,6a,8ß)-form, [4-Hydroxy-2-(4-hydroxy-2-hydroxymethyl-2E-butenoyloxymethyl)-2E-butenoyl]	C25H30O9
		8-Hydroxy-3,10(14),11(13)-guaiatrien-12,6-olide; (1a,5a,6a,8ß)-form, 8-[4-Hydroxy-2-(4-hydroxy-2-hydroxymethyl-2-butenoyloxymethyl)-2-butenoyl](E,E-)	C25H30O9
		Isocryptochlorophaeic acid; 4-Me ether	C26H34O8
		4-Isopropylbenzyl alcohol; O-[ß-D-Glucopyranosyl-(1?2)-ß-D-galactopyranoside]	C22H34O11
		2-Isopropyl-5-methyl-1,4-benzenediol; 5-O-[a-L-Rhamnopyranosyl-(1?2)-ß-D-glucopyranoside]	C22H34O11
		2-Isopropyl-5-methylphenol; O-[ß-D-Glucopyranosyl-(1?2)-ß-D-glucopyranoside]	C22H34O11
		5-Isopropyl-2-methylphenol; O-[ß-D-Glucopyranosyl-(1?2)-ß-D-glucopyranoside]	C22H34O11
		Kadsulignan A; 6ß-Acetoxy	C25H30O9
		Kopsaporine; 12-Methoxy, 14,15-dihydro, 15a-hydroxy	C24H30N2O8
		Ligulaverin C; 6-Deacyl, 6-angeloyl	C26H34O8
		Loxodinol	C25H30O9
		Maximowicziol A; 11-Ketone, 4-O-ß-D-glucopyranoside	C25H30O9
		Munronin F	C25H30O9
		Murrafoline B	C32H30N2O2
		Murrafoline B; (±)-form	C32H30N2O2
		Murrafoline D	C32H30N2O2
		Murrafoline D; (±)-form	C32H30N2O2
		Murrafoline G	C32H30N2O2
		Murrafoline G; (±)-form	C32H30N2O2
		Murrastifoline D; 3'-Deoxy	C32H30N2O2
		1,3,5,14,19-Pentahydroxybufa-20,22-dienolide; (1ß,3ß,5ß,14ß)-form, 19-Aldehyde, 3-Ac	C26H34O8
		3,6,8,12,14-Pentahydroxybufa-4,20,22-trienolide; (3ß,6ß,12ß,14ß)-form, 6-Ac	C26H34O8
		1,3,7,11,12-Pentahydroxy-14-meliacen-28-oic acid; (1a,3a,7a,11ß,12a)-form, 7-Ketone	C26H34O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
473.201 (continued)	**100**	Picrasin A	C26H34O8
		Podophyllic acid; (7R,7'R,8R,8'R)-form, N-Ethylhydrazide	C24H30N2O8
		Robustaol A	C25H30O9
		Saroaspidin C	C26H34O8
		4,15-Scirpenediol; 4ß-form, Dibenzoyl	C29H30O6
		13(16),14-Spongiadiene-2,3,17,19-tetrol; 3a-form, 2-Ketone, tri-Ac	C26H34O8
		Terretonin D	C26H34O8
		2,4,6,8,10,12-Tetradecahexaenedioic acid; (all-E)-form, 1-L-Aspartic acid, 14-L-isoleucine diamide	C24H30N2O8
		1,4,6,9-Tetrahydroxydihydro-ß-agarofuran; (1a,4ß,6ß,9ß)-form, 1-Benzoyl, 6,9-di-Ac	C26H34O8
		1,6,8,9-Tetrahydroxydihydro-ß-agarofuran; (1a,6ß,8a,9a)-form, 9-Benzoyl, 1,6-di-Ac	C26H34O8
		3,5,11,14-Tetrahydroxy-12-oxobufa-20,22-dienolide; (3ß,5ß,11a,14ß)-form, 3-Ac	C26H34O8
		3,5,12,14-Tetrahydroxy-11-oxobufa-20,22-dienolide; (3ß,5ß,12ß,14ß)-form, 3-Ac	C26H34O8
		2,3,14-Trihydroxy-5,8(17),11-briaratrien-18,7-olide; (2ß,3a,5Z,7a,14a)-form, Tri-Ac	C26H34O8
		6,10,14-Trihydroxy-3,7,11,15(17)-cembratetraen-16,2-olide; (1S,2S,3E,6S,7E,10S,11E,14S)-form, Tri-Ac	C26H34O8
		5,6,8-Trihydroxy-28-norisotoonafolin	C25H30O9
		5,6,8-Trihydroxy-28-norisotoonafolin; (5a,6ß,8a)-form	C25H30O9
		2,11,12-Trihydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial 11,6-hemiacetal; 2a-form, Tri-Ac(6a-)	C26H34O8
		Trillenogenin; 1-Ketone	C26H34O8
		14(17)-Vouacapene-1,5,6,7-tetrol; (1a,5a,6a,7ß)-form, 1,6,7-Tri-Ac	C26H34O8
		Xenione	C26H34O8
		Xenione; Stereoisomer	C26H34O8
517.161	**1000**		
		(2-Acetamido-2-deoxy-a-D-glucopyranos-1-yl)(methyl 2-acetamido-2-deoxy-a-D-glucopyranosid-6-yl) phosphate	C17H31N2O14P
		Acylsucroses; 3'-O-(4-Hydroxy-3-methoxy-E-cinnamoyl)	C22H30O14
		Acylsucroses; 6-O-(4-Hydroxy-3-methoxy-E-cinnamoyl)	C22H30O14
		Ajmaline, BAN, JAN; N-Propyl, hydrogen tartrate	C27H38N2O8
		Alangium Alkaloid AL64; Mono-Ac	C31H39N3O4
		Allaric acid; D-form, 1,4-Lactone, 2,3,5-tribenzoyl, 6-Me ester	C28H22O10
		Aloin, BAN; (10R)-form, 7-Hydroxy, 4',6'-di-Ac	C25H26O12
		Aloin, BAN; (10S)-form, 7-Hydroxy, 4',6'-di-Ac	C25H26O12
		Ambruticin VS 5; N,N-Di-Me, N-oxide	C30H47NO6
		Antibiotic GKK 1032A2; 2'-Me ether	C33H43NO4
		Antibiotic TAN 1532B; 13-Me ether	C30H30O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161 (continued)	**1000**	Austalide H; 13-Ac	C28H38O9
		Batrachotoxinin A; O20-(3-Hydroxypentanoyl)	C29H43NO7
		Bisnubenolide	C30H30O8
		Cedrodorin; 9a-Hydroxy	C27H34O10
		Chlorodesmin	C28H38O9
		Colletotrichin A; 3-O-Formyl	C29H42O8
		Colletotrichin A; 3"-O-Formyl	C29H42O8
		Cosmosiin; 6"-O-Malonyl	C24H22O13
		Cyclo(alanylalanylvalyltyrosylglycylglycyl)	C24H34N6O7
		8-Daucene-2,4,6,10-tetrol; (2a,4ß,6a,10a)-form, 6-O-(3,4-Dimethoxybenzoyl), 2,10-di-Ac	C28H38O9
		Deacetylasperuloside; 10-O-(4-Hydroxy-E-cinnamoyl)	C25H26O12
		Deacetylasperuloside; 10-O-a-L-Rhamnopyranoside	C22H30O14
		Dehydrobruceolide; 15-O-(3-Methyl-2-butenoyl)	C26H30O11
		ß-D-Galactopyranosyl-(1?6)-ß-D-galactopyranosyl-(1?6)-D-galactose, 9CI; ß-Pyranose-form, Me glycoside	C19H34O16
		a-D-Galactopyranosyl-(1?3)-[a-D-galactopyranosyl-(1?6)]-D-glucose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		a-D-Galactopyranosyl-(1?4)-ß-D-galactopyranosyl-(1?4)-D-glucose, 9CI; ß-Pyranose-form, Me glycoside	C19H34O16
		a-D-Galactopyranosyl-(1?2)-[ß-D-glucopyranosyl-(1?3)]-D-glucose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		a-D-Galactopyranosyl-(1?3)-[ß-D-glucopyranuronosyl-(1?2)]-D-mannose, 9CI	C18H30O17
		a-D-Galactopyranosyl-(1?3)-[ß-D-glucopyranuronosyl-(1?2)]-D-mannose, 9CI; a-Pyranose-form	C18H30O17
		a-D-Galactopyranosyl-(1?3)-[a-D-mannopyranosyl-(1?6)]-D-mannose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		ß-D-Glucopyranosyl-(1?3)-a-D-galactopyranuronosyl-(1?3)-D-mannose, 9CI	C18H30O17
		ß-D-Glucopyranosyl-(1?2)-[ß-D-glucopyranosyl-(1?6)]-D-glucose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		a-D-Glucopyranosyl-(1?3)-a-D-glucopyranosyl-(1?3)-D-glucose, 8CI; ß-Pyranose-form, Me glycoside	C19H34O16
		a-D-Glucopyranosyl-(1?3)-a-D-glucopyranosyl-(1?4)-D-glucose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		ß-D-Glucopyranosyl-(1?3)-[ß-D-glucopyranosyl-(1?6)]-D-glucose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		ß-D-Glucopyranosyl-(1?3)-[ß-D-glucopyranosyl-(1?6)]-D-glucose, 9CI; ß-Pyranose-form, Me glycoside	C19H34O16
		a-D-Glucopyranosyl-(1?4)-a-D-glucopyranosyl-(1?3)-D-glucose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		a-D-Glucopyranosyl-(1?4)-[a-D-glucopyranosyl-(1?6)]-D-glucose, 9CI; ß-Pyranose-form, Me glycoside	C19H34O16
		a-D-Glucopyranosyl-(1?2)-a-D-glucopyranuronosyl-(1?3)-D-mannose, 9CI	C18H30O17

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	a-D-Glucopyranosyl-(1?6)-[a-D-mannopyranosyl-(1?3)]-D-mannose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
(continued)		ß-D-Glucopyranuronosyl-(1?6)-ß-D-galactopyranosyl-(1?6)-D-galactose, 9CI	C18H30O17
		ß-D-Glucopyranuronosyl-(1?4)-ß-D-glucopyranosyl-(1?4)-D-glucose	C18H30O17
		a-D-Mannopyranosyl-(1?4)-ß-D-glucopyranuronosyl-(1?2)-D-mannose, 9CI	C18H30O17
		a-D-Mannopyranosyl-(1?2)-[a-D-mannopyranosyl-(1?4)]-D-mannose, 9CI; a-Pyranose-form, Me glycoside	C19H34O16
		ß-D-Mannopyranuronosyl-(1?4)-ß-D-glucopyranosyl-(1?4)-D-galactose, 8CI	C18H30O17
		6-O-ß-D-Xylopyranosyl-D-galactose, 9CI; a-Pyranose-form, 1,2:3,4-Di-O-isopropylidene, tri-Ac	C23H34O13
		3,4-Di-O-caffeoylquinic acid; 7",8"-Dihydro	C25H26O12
		6,6'-Dichloroindigotin; N-(4-Acetamido-4,6-dideoxy-ß-D-glucopyranosyl)	C24H21Cl2N3O6
		Didethiodi(methylthio)acetylapoaranotin	C24H26N2O7S2
		2,3:6,7-Diepoxy-1,5,12,16,27-pentahydroxywith-24-enolide; (1a,2a,3a,5a,6a,7a,12a,16ß,22R)-form, 16-Ketone	C28H38O9
		4,18:7,20-Diepoxy-15,19,20-trihydroxy-6-oxo-11-cleroden-16,15-olide; (ent-4ß,5ß,7ß,11E,15?, 20R)-form, Tri-Ac	C26H30O11
		3-[9,10-Dihydro-3,4,7,9-tetrahydroxy-10-(hydroxymethyl)-2-phenanthrenyl]-2-propenal; 3,7-Di-Me ether, 4-O-ß-D-glucopyranoside	C26H30O11
		4,5-Dihydroxyanthraquinone-2-carboxylic acid; 5-O-(6-O-Oxalyl-ß-D-glucopyranoside)	C23H18O14
		3,14-Dihydroxycarda-4,20(22)-dienolide; (3ß,14ß)-form, 3-O-a-L-Rhamnopyranoside	C29H42O8
		3,14-Dihydroxycard-20(22)-enolide; (3ß,5a,14ß,17a)-form, 3-O-(4-Amino-2,4,6-trideoxy-3-O-methyl-ß-D-ribo-hexopyranoside)	C30H47NO6
		3,14-Dihydroxycard-20(22)-enolide; (3ß,5a,14ß,17a)-form, 3-O-(4-Amino-2,4,6-trideoxy-3-O-methyl-a-L-arabino-hexopyranoside)	C30H47NO6
		5,7-Dihydroxy-4',6-dimethoxyflavone; 7-O-(6-O-Acetyl-ß-D-glucopyranoside)	C25H26O12
		1,2-Dihydroxy-3,11(13)-eudesmadien-12,8-olid-14-oic acid; (1a,2ß,8ß)-form, Di-Ac, 4,5-dimethyl-3-oxo-2-heptyl ester	C28H38O9
		3,8-Dihydroxy-1(10),4,11(13)-germacratrien-12,6-olide; (1(10)E,3ß,4Z,6a,8ß)-form, 8-O-[2-[(4-Hydroxy-2-hydroxymethyl-2E-butenoyloxy)methyl]-2E-butenoyl], 3-Ac	C27H34O10

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	3,8-Dihydroxy-1(10),4,11(13)-germacratrien-12,6-olide; (1(10)E,3ß,4Z,6a,8ß)-form, 8-O-[4-Hydroxy-2-(2-hydroxymethyl-2E-butenoyloxy)methyl-2E-butenoyl], 3-Ac	C27H34O10
(continued)		3,5-Dihydroxy-6,7-megastigmadien-9-one; (3S,5R,7Rax)-form, 3-O-[ß-D-Apiofuranosyl-(1?6)-ß-D-glucopyranoside]	C24H38O12
		6,9-Dihydroxy-4,7-megastigmadien-3-one; (6S,7E,9R)-form, 9-O-[ß-D-Apiofuranosyl-(1?2)-ß-D-glucopyranoside]	C24H38O12
		6,9-Dihydroxy-4,7-megastigmadien-3-one; (6S,7E,9R)-form, 9-O-[ß-D-Apiofuranosyl-(1?6)-ß-D-glucopyranoside]	C24H38O12
		6,9-Dihydroxy-4,7-megastigmadien-3-one; (6?,7E,9?)-form, 9-O-[ß-D-Apiofuranosyl-(1?6)-ß-D-glucopyranoside]	C24H38O12
		6,9-Dihydroxy-4,7-megastigmadien-3-one; (6S,7E,9R)-form, 9-O-[ß-D-Xylopyranosyl-(1?6)-ß-D-glucopyranoside]	C24H38O12
		6,9-Dihydroxy-4,7-megastigmadien-3-one; (6S,7E,9R)-form, 9-O-[a-L-Arabinopyranosyl-(1?6)-ß-D-glucopyranoside]	C24H38O12
		7,18-Dihydroxy-4-methylaconit-8(15)-en-14-one; 5ß-form, O7-Me, N-Et, 18-O-(2-acetamidobenzoyl)	C31H38N2O5
		19,22-Dihydroxy-24-nor-2,3-seco-4,12-ursadiene-2,3,28-trioic acid	C29H42O8
		19,22-Dihydroxy-24-nor-2,3-seco-4,12-ursadiene-2,3,28-trioic acid; (4Z,19a,22a)-form	C29H42O8
		3,14-Dihydroxy-19-oxocarda-4,20(22)-dienolide; (3ß,14ß)-form, 3-O-ß-D-Xylopyranoside	C28H38O9
		3,14-Dihydroxy-19-oxocard-20(22)-enolide; (3ß,5a,14ß)-form, 3-O-(2,6-Dideoxy-D-ribo-hexopyranoside)	C29H42O8
		3,14-Dihydroxy-19-oxocard-20(22)-enolide; (3ß,5a,14ß,17a)-form, 3-O-(2,6-Dideoxy-D-ribo-hexopyranoside)	C29H42O8
		3,14-Dihydroxy-19-oxocard-20(22)-enolide; (3ß,5ß,14ß)-form, 3-O-(2,6-Dideoxy-D-ribo-hexopyranoside)	C29H42O8
		3,14-Dihydroxy-19-oxocard-20(22)-enolide; (3ß,5a,14ß)-form, 3-O-(2,6-Dideoxy-D-xylo-hexopyranoside)	C29H42O8
		3,14-Dihydroxy-19-oxocard-20(22)-enolide; (3a,5a,14ß)-form, 3-O-(2,6-Dideoxy-D-xylo-hexopyranoside)	C29H42O8
		3,14-Dihydroxy-19-oxocard-20(22)-enolide; (3a,5a,14ß,17a)-form, 3-O-(2,6-Dideoxy-D-xylo-hexopyranoside)	C29H42O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	3-(3,4-Dihydroxyphenyl)-2-propen-1-ol; (E)-form, 1,3'-Di-Me ether, 4'-O-[3,4-dihydroxy-E-cinnamoyl-(?6)-ß-D-glucopyranoside]	C26H30O11
(continued)		Echitamidine; (-)-form, Nb-Oxide, 19-O-ß-D-glucopyranoside	C26H34N2O9
		4,7'-Epoxy-3,8'-bilign-7-ene-3',4',5,9,9'-pentol; (7'R,8'S)-form, 9-Aldehyde, 3',5-di-Me ether, 4'-O-ß-D-glucopyranoside	C26H30O11
		4,7'-Epoxy-3,8'-bilign-7-ene-3',4',5,9,9'-pentol; (7'S,8'R)-form, 9-Aldehyde, 3',5-di-Me ether, 4'-O-ß-D-glucopyranoside	C26H30O11
		4,7'-Epoxy-3,8'-bilign-7-ene-3',4',5,9,9'-pentol; (7'R,8'S)-form, 9-Aldehyde, 3',5-di-Me ether, 9'-O-ß-D-glucopyranoside	C26H30O11
		4,7'-Epoxy-3,8'-bilign-7-ene-3',4',5,9,9'-pentol; (7'S,8'R)-form, 9-Aldehyde, 3',5-di-Me ether, 9'-O-ß-D-glucopyranoside	C26H30O11
		18,19-Epoxy-12,14-clerodadiene-2,6,18,19-tetrol; (ent-2ß,5a,6a,8a,12E,18a,19a)-form, 2-Tigloyl, 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,12,14-clerodatriene-2,6,7,18,19-pentol; (2a,5a,6a,7ß,8a,12Z,18a,19a)-form, 2-Ketone, 7-butanoyl, 18,19-di-Ac	C28H38O9
		18,19-Epoxy-3,12,14-clerodatriene-2,6,18,19-tetrol; (ent-2ß,5a,6a,8a,12E,18a,19a)-form, 6-Me ether, 2-O-(2-methylpropanoyl), 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,12,14-clerodatriene-2,6,18,19-tetrol; (2ß,5a,6a,8a,12E,18a,19a)-form, 2-O-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,12,14-clerodatriene-2,6,18,19-tetrol; (ent-2ß,5a,6a,8a,12E,18a,19a)-form, 2-O-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,12,14-clerodatriene-2,6,18,19-tetrol; (2a,5a,6a,8a,12E,18a,19a)-form, 2-O-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,12,14-clerodatriene-2,6,18,19-tetrol; (ent-2a,5a,6a,8a,12E,18a,19a)-form, 6-O-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,12,14-clerodatriene-2,7,18,19-tetrol; (2a,5a,7ß,8a,12Z,18a,19a)-form, 2-Me ether, 7-butanoyl, 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,13(16),14-clerodatriene-2,6,18,19-tetrol; (2a,5a,6a,8ßH,18a,19ß)-form, 6-Me ether, 2-(2-methylpropanoyl), 18,19-di-Ac	C29H42O8
		18,19-Epoxy-3,13(16),14-clerodatriene-2,6,18,19-tetrol; (2a,5a,6a,8ßH,18a,19ß)-form, 2-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	18,19-Epoxy-3,13(16),14-clerodatriene-2,6,18,19-tetrol; (ent-2a,5a,6a,8ßH,18a,19ß)-form, 2-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8
(continued)		18,19-Epoxy-3,13(16),14-clerodatriene-2,6,18,19-tetrol; (ent-2ß,5a,6a,8ßH,18a,19a)-form, 2-(2-Methylbutanoyl), 18,19-di-Ac	C29H42O8
		11,12-Epoxy-2,3,14-trihydroxy-5,8(17)-briaradien-18,7-olide; (2ß,3a,7a,11ß,12ß,14a)-form, 2-Butanoyl, 3,14-di-Ac	C28H38O9
		11,12-Epoxy-2,4,14-trihydroxy-5,8(17)-briaradien-18,7-olide; 2-Butanoyl, 4,14-di-Ac	C28H38O9
		16,24-Epoxy-21,24,25-trihydroxy-17-cheilanthen-19,25-olide; (24a,25?)-form, 21,24-Di-Ac	C29H42O8
		5,6-Epoxy-3,4,28-trihydroxy-1-oxowith-24-enolid-19-oic acid	C28H38O9
		5,6-Epoxy-3,4,28-trihydroxy-1-oxowith-24-enolid-19-oic acid; (3ß,4ß,5ß,6ß)-form	C28H38O9
		17,18-Epoxy-1(19),6,10(17),13-xenicatetraene-8,11,12,18-tetrol; (6E,8a,11R,12R,18ß)-form, Tetra-Ac	C28H38O9
		Euchretin C; 9-Me ether	C31H34O7
		Fibleucin; 4-O-ß-D-Glucopyranoside	C26H30O11
		Fumadensine	C30H34N2O6
		Galactinol; 6'-O-a-D-Galactopyranosyl, 4-Me	C19H34O16
		Galactotriose; ß-Pyranose-form, Me glycoside	C19H34O16
		Gentiopicroside; 3'-ß-D-Glucopyranosyl	C22H30O14
		Gentiopicroside; 4'-ß-D-Glucopyranosyl	C22H30O14
		Gentiopicroside; 6'-ß-D-Glucopyranosyl	C22H30O14
		8-Glucopyranosyl-4',5,7-trihydroxyisoflavone; 6"-O-Malonyl	C24H22O13
		Gomphoside	C29H42O8
		Gomphotin	C29H42O8
		Gossypol	C30H30O8
		Gossypol; (+)-form	C30H30O8
		Gossypol; (-)-form	C30H30O8
		Gossypol; (±)-form	C30H30O8
		Guan-fu base E	C29H43NO7
		Haperforin D	C27H34O10
		2,3',4,4',5',6-Hexahydroxydiphenyl ether; Hexa-Ac	C24H22O13
		2,3,14,20,22,28-Hexahydroxy-6-oxostigmast-7-en-26-oic acid; (2ß,3ß,5ß,14a,20R,22R,24S,25S,28R)-form, 26?28-Lactone, 22-ketone	C29H42O8
		9-Hydroxy-4-megastigmen-3-one; (6R,9R)-form, O-[a-L-Rhamnopyranosyl-(1?6)-ß-D-glucopyranoside]	C25H42O11
		1-Hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone, 9CI; Fe complex (3:1)	C21H24FeN3O9
		3-Hydroxy-9-methoxypterocarpan; (-)-form, 3-O-(6-O-Malonyl-ß-D-glucopyranoside)	C25H26O12
		10-Hydroxyoleoside; (E)-form, 6',10-Di-Ac, di-Me ester	C22H30O14

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	chiro-Inositol, 9CI, 8CI; D-form, 4-Me, 2-O-[a-D-galactopyranosyl-(1?6)-a-D-galactopyranoside]	C19H34O16
(continued)		myo-Inositol, 8CI, 9CI; 2-O-[a-D-Mannopyranosyl-(1?4)-a-D-glucuronopyranoside]	C18H30O17
		Ipobscurine B	C29H30N2O7
		Ixoside; 6'-(E-Cinnamoyl)	C25H26O12
		Jioglutoside B	C23H34O13
		16-Kaurene-3,6,7,11,15-pentol; (ent-3a,6ß,7a,11a,15a)-form, 11-Ketone, tetra-Ac	C28H38O9
		16-Kaurene-3,6,7,11,15-pentol; (ent-3a,6ß,7a,11a,15a)-form, 15-Ketone, tetra-Ac	C28H38O9
		16-Kaurene-3,6,7,11,15-pentol; (ent-3a,6ß,7a,11a,15a)-form, 6-Ketone, tetra-Ac	C28H38O9
		Khayanolide B	C27H34O10
		Lebstatin; 17-Demethoxy	C28H42N2O7
		2-O-a-L-Fucopyranosyl-D-galactose; ß-Pyranose-form, 1,6-Anhydro, penta-Ac	C22H30O14
		Lotusine A	C30H38N4O4
		Maculalactone E; 3a,3aa-Epoxide	C34H30O5
		Mallotus B	C30H30O8
		5,7-Megastigmadiene-3,4,9-triol; (3?,4?,7E)-form, 9-Ketone, 4-O-[ß-D-apiofuranosyl-(1??)-ß-D-glucopyranoside]	C24H38O12
		5-Megastigmene-3,9-diol; (3ß,9R)-form, 9-Ketone, 3-O-[a-L-rhamnopyranosyl-(1?6)-ß-D-glucopyranoside]	C25H42O11
		Mitiphylline; N-De-Me	C30H47NO6
		Myricanol; 11-Deoxy, 9-oxo, 5-O-ß-D-glucopyranoside	C27H34O10
		Myricanol; Ketone, 5-O-ß-D-glucopyranoside	C27H34O10
		1,4,5-Naphthalenetriol, 9CI; 4-O-[4-Hydroxy-3,5-dimethoxybenzoyl-(?6)-ß-D-glucopyranoside]	C25H26O12
		3,3',4,4',5,5',9,9'-Octahydroxy-7,7'-epoxylignan; (7R,7'S,8R,8'R)-form, 3',4'-Methylene, 3,5,5'-tri-Me ether, 9,9'-di-Ac	C26H30O11
		3,3',4,4',5,5',9,9'-Octahydroxylignan; (8S,8'S)-form, 3,4-Methylene, 3',4',5,5'-tetra-Me ether, 9,9'-di-Ac	C27H34O10
		Opuntial	C28H38O9
		Paspalitrem A; ?1'-Isomer, 3'-hydroxy	C32H39NO5
		1,1,2,6,6-Pentabromo-1,4-octadien-3-one	C8H7Br5O
		1,1,2,6,6-Pentabromo-1,4-octadien-3-one; (E)-form	C8H7Br5O
		3,4',5,7,8-Pentahydroxyflavone; 8-O-(2,3-Di-O-acetyl-ß-D-xylopyranoside)	C24H22O13
		2,3,14,21,22-Pentahydroxy-6-oxoergosta-7,24(28)-dien-29,25-olide	C29H42O8
		2,3,14,21,22-Pentahydroxy-6-oxoergosta-7,24(28)-dien-29,25-olide; (2ß,3ß,5ß,14a,20R,22R)-form	C29H42O8
		2,3,14,20,25-Pentahydroxy-6-oxostigmasta-7,24(28)-dien-29,22-olide	C29H42O8
		2,3,14,20,25-Pentahydroxy-6-oxostigmasta-7,24(28)-dien-29,22-olide; (2ß,3ß,5ß,14a,20R,22R)-form	C29H42O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	2,3,14,20,28-Pentahydroxy-6-oxostigmasta-7,24-dien-26,22-olide	C29H42O8
(continued)		2,3,14,20,28-Pentahydroxy-6-oxostigmasta-7,24-dien-26,22-olide; (2ß,3ß,5ß,14a,20R,22R,28?)-form	C29H42O8
		Petrosaspongiolide G; 16,21-Di-Ac	C29H42O8
		Petrosaspongiolide G; 17,24,25-Triepimer, 16,21-di-Ac	C29H42O8
		Phellamuretin; 3-O-ß-D-Glucopyranoside	C26H30O11
		Pinillidine	C28H22O10
		Piperitol; (+)-form, O-ß-D-Glucopyranoside	C26H30O11
		Polybromohydroxydiphenyl ethers; 2,2',4,4'-Tetrabromo-6,6'-dihydroxydiphenyl ether	C12H6Br4O3
		Polybromohydroxydiphenyl ethers; 2,3',4,5'-Tetrabromo-2',6-dihydroxydiphenyl ether	C12H6Br4O3
		Polybromohydroxydiphenyl ethers; 3,3',5,6'-Tetrabromo-2,2'-dihydroxydiphenyl ether	C12H6Br4O3
		Polybromohydroxydiphenyl ethers; 3,5,5',6-Tetrabromo-2,2'-dihydroxydiphenyl ether	C12H6Br4O3
		5-(1-Propenyl)-1,2,3-benzenetriol, 9CI; (E)-form, 1,3-Di-Me ether, 2-O-[ß-D-glucopyranosyl-(1?6)-ß-D-glucopyranoside]	C23H34O13
		5-(2-Propenyl)-1,2,3-benzenetriol; 1,3-Di-Me ether, 2-O-[ß-D-glucopyranosyl-(1?6)-ß-D-glucopyranoside]	C23H34O13
		Purealidin H; O4-(3-Aminopropyl)	C17H22Br2N6O3
		Quercetin 3-glycosides; Monosaccharides, 3-O-(3,5-Di-O-acetyl-a-L-arabinofuranoside)	C24H22O13
		Rubratoxin A; 25-Ketone	C26H30O11
		Rutaevinexic acid	C26H30O11
		Sarcotrine A; N-Dealkyl, N-(2-phenylethyl)	C33H43NO4
		Sarcotrine A; 17-Epimer, N-dealkyl, N-(2-phenylethyl)	C33H43NO4
		Sativanine B	C30H38N4O4
		Scandenin; Di-Ac	C30H30O8
		Scytonine	C31H22N2O6
		Segetalin G	C25H38N6O6
		Sirodesmin A; Trisulfide homologue	C20H26N2O8S3
		Sirodesmin A; Trisulfide homologue, 1-epimer	C20H26N2O8S3
		13(16),14-Spongiadiene-2,3,17,19-tetrol; (2ß,3ß)-form, Tetra-Ac	C28H38O9
		Swertiabisxanthone I	C26H14O12
		4(20),11-Taxadiene-2,5,10,14-tetrol; (2a,5a,10ß,14ß)-form, 14-Propanoyl, 2,5,10-tri-Ac	C29H42O8
		Terpendole C; 6,7-Didehydro	C32H39NO5
		Terragine A	C26H38N4O7
		1,4,9,20-Tetraacetoxy-1,3(20),6,10,14-phytapentaen-19-al	C28H38O9
		2,3,11,15-Tetrahydroxy-16-kauren-6-one; (ent-2a,3a,11a,15a)-form, Tetra-Ac	C28H38O9

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
517.161	**1000**	2,3,6,11-Tetrahydroxy-16-kauren-15-one; (ent-2a,3a,6ß,11ß)-form, Tetra-Ac	C28H38O9
(continued)		4,14,17,20-Tetrahydroxy-1-oxowitha-2,5,24-trienolide; (4ß,14a,17ßOH,20S,22R)-form, 28-Hydroxy, 5ß,6ß-epoxide	C28H38O9
		5,6,14,15-Tetrahydroxy-1-oxowitha-2,16,24-trienolide; (5a,6ß,14a,15a,22R)-form, 25-Hydroxy, 16,17-epoxide	C28H38O9
		3,4',5,7-Tetrahydroxy-3'-prenylflavanone; (2R,3R)-form, 7-O-ß-D-Glucopyranoside	C26H30O11
		3,4',5,7-Tetrahydroxy-8-prenylflavanone; (2R,3R)-form, 7-O-ß-D-Glucopyranoside	C26H30O11
		2,5,9,10-Tetrahydroxy-4(20),11-taxadien-13-one; (2a,5a,9a,10ß)-form, Tetra-Ac	C28H38O9
		Thevetiogenin; 3-O-a-L-Rhamnopyranoside	C29H42O8
		5,7,8-Trihydroxy-2H-1-benzopyran-2-one, 9CI; 5-O-[ß-D-Glucopyranosyl-(1?6)-ß-D-glucopyranoside]	C21H26O15
		5,7,8-Trihydroxy-2H-1-benzopyran-2-one, 9CI; 5,7-Di-O-ß-D-glucopyranoside	C21H26O15
		4',5,7-Trihydroxyflavone; 7-O-(2-ß-D-Glucuronopyranosyloxypropanoyl)	C24H22O13
		4',5,7-Trihydroxyflavone; 5-O-(6-O-Malonyl-ß-D-glucopyranoside)	C24H22O13
		5,6,7-Trihydroxyflavone; 7-O-(6-O-Malonyl-ß-D-glucopyranoside)	C24H22O13
		4',5,7-Trihydroxyisoflavone; 7-O-(6-O-Malonyl-D-glucopyranoside)	C24H22O13
		1,2,8-Trihydroxy-3-methylanthraquinone; 2-O-(6-O-Malonyl-ß-D-glucopyranoside)	C24H22O13
		4',5,7-Trihydroxy-8-prenylflavanone; (S)-form, 4"-Hydroxy(E-), 7-O-ß-D-glucopyranoside	C26H30O11
		4',5,7-Trihydroxy-8-prenylisoflavone; 2",3"-Dihydro, 3"-hydroxy, 7-O-ß-D-glucopyranoside	C26H30O11
		3,7,12-Trihydroxy-25,26,27-trinor-11,15-dioxolanost-8-en-24-oic acid; (3ß,7ß,12ß)-form, 12-Ac	C29H42O8
		Trioxacarcin A, 9CI; Aglycone	C25H26O12
		Tryptoquialanine A	C27H26N4O7
		Ustilaginoidin A; (R)-form, 2?,2'?,3,3'-Tetrahydro	C28H22O10
		Ustilaginoidin A; (S)-form, 2?,2'?,3,3'-Tetrahydro	C28H22O10
		Venturamide B	C22H26N6O5S2
		Veprisonic acid	C27H34O10
		Veprisonic acid; 21-Ketone(lactone), 23?-alcohol	C27H34O10
		Verrucarin A; 16-Hydroxy	C27H34O10
		Verrucarin A; 8-Hydroxy	C27H34O10
		Xanthomonadin I	C24H22Br2O3

Table A.2. Putative matches for Compound X from the Chapman & Hall database based on m/z ratios obtained from FTMS analysis of pooled Compound X fractions from HPLC of calf urine.

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
317.0958	**700**		
		2-Acetyl-1,3,6,8-tetrahydroxyanthraquinone; 1'R-Alcohol	C16H12O7
		2-Amino-3-(3-bromo-4-hydroxyphenyl)propanoic acid; (S)-form, 4-Me ether, N,N,N-tri-Me, betaine	C13H18BrNO3
		1,5-Anhydroglucitol, 9CI, 8CI; D-form, 2-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		1,5-Anhydroglucitol, 9CI, 8CI; D-form, 6-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		Aquilegiolide; 4,5-Dihydro, O-ß-D-glucopyranoside	C14H20O8
		Aquilegiolide; 6-Epimer, 4,5-dihydro, O-ß-D-glucopyranoside	C14H20O8
		Aquilegiolide; 7a-Epimer, 4,5-dihydro, O-ß-D-glucopyranoside	C14H20O8
		1,2,3-Benzenetriol, 9CI; 1,3-Di-Me ether, 2-O-ß-D-glucopyranoside	C14H20O8
		1,2,4-Benzenetriol, 8CI, 9CI; 1,2-Di-Me ether, 4-O-ß-D-glucopyranoside	C14H20O8
		1,3,5-Benzenetriol, 9CI; Di-Me ether, O-ß-glucopyranoside	C14H20O8
		Boxazomycin A	C14H12N4O5
		6-Bromo-1,2,3,4-tetrahydro-7,8-dihydroxy-3-isoquinolinecarboxylic acid; (S)-form, 7-Me ether, Me ester	C12H14BrNO4
		8-Bromo-1,2,3,4-tetrahydro-6,7-dihydroxy-3-isoquinolinecarboxylic acid; (S)-form, 7-Me ether, Me ester	C12H14BrNO4
		Coccineone D	C16H12O7
		Crombeone	C16H12O7
		1,2,3,4-Cyclohexanetetrol, 9CI; (1RS,2RS,3RS,4RS)-form, Tetra-Ac	C14H20O8
		1,2,4,5-Cyclohexanetetrol, 9CI; (1S,2S,4S,5S)-form, Tetra-Ac	C14H20O8
		1,1-Dibromo-2-heptanol; (±)-form, Ac	C9H16Br2O2
		2',5'-Dihydroxyacetophenone, 8CI; Bis(2,2,2-trifluoroethyl) ether	C12H10F6O3
		2,3-Dihydroxybenzoic acid, 9CI; 3-O-ß-D-Glucopyranoside	C13H16O9
		2,5-Dihydroxybenzoic acid, 9CI; 2-O-ß-D-Glucopyranoside	C13H16O9
		2,5-Dihydroxybenzoic acid, 9CI; 5-O-ß-D-Glucopyranoside	C13H16O9
		3,4-Dihydroxybenzoic acid; 3-O-ß-D-Glucopyranoside	C13H16O9
		3,4-Dihydroxybenzoic acid; 4-O-ß-D-Glucopyranoside	C13H16O9

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
317.0958	**700**	3,4-Dihydroxybenzyl alcohol, 8CI; 3-Me ether, 4-O-ß-D-glucopyranoside	C14H20O8
(continued)		3-(3,4-Dihydroxybenzylidene)-5,6,7-trihydroxy-4-chromanone	C16H12O7
		3-(3,4-Dihydroxybenzylidene)-5,6,7-trihydroxy-4-chromanone; (Z)-form	C16H12O7
		3-(3,4-Dihydroxybenzylidene)-5,7,8-trihydroxy-4-chromanone	C16H12O7
		3-(3,4-Dihydroxybenzylidene)-5,7,8-trihydroxy-4-chromanone; (Z)-form	C16H12O7
		1,9-Dihydroxy-3-hydroxymethyl-10-methoxydibenz[b,e]oxepin-6,11-dione	C16H12O7
		2,8-Dihydroxy-6-hydroxymethylxanthone-1-carboxylic acid; Me ester	C16H12O7
		2,8-Dihydroxy-6-hydroxymethylxanthone-1-carboxylic acid; 8-Me ether	C16H12O7
		2-(3,4-Dihydroxyphenyl)-4,6-dihydroxy-3-benzofurancarboxylic acid; Me ester	C16H12O7
		2-(3,4-Dihydroxyphenyl)ethanol; 3'-O-ß-D-Galactopyranoside	C14H20O8
		2-(3,4-Dihydroxyphenyl)ethanol; 3'-O-ß-D-Glucopyranoside	C14H20O8
		2-(3,4-Dihydroxyphenyl)ethanol; 4'-O-ß-D-Glucopyranoside	C14H20O8
		2-(3,5-Dihydroxyphenyl)ethanol; 3'-O-ß-D-Glucopyranoside	C14H20O8
		1-(2,4-Dihydroxyphenyl)-2-(2,4,5-trihydroxyphenyl)ethanedione; 4,5-Methylene, 4'-Me ether	C16H12O7
		Ellagic acid, INN; 3-Me ether	C15H8O8
		1,2-Epoxy-8-hydroxy-6-(hydroxymethyl)-1-methoxycarbonylxanthone	C16H12O7
		Fusidienol	C16H12O7
		altro-2-Heptulose, 8CI, 9CI; ß-D-Pyranose-form, 2,7-Anhydro, 4,5-O-isopropylidene, 1,3-di-Ac	C14H20O8
		4-Hydroxy-4-(2-hydroxyethyl)-2,5-cyclohexadien-1-one; 2'-O-ß-D-Glucopyranoside	C14H20O8
		4-Hydroxy-2-isopropylidene-5-methyl-3(2H)-furanone; O-ß-D-Glucopyranoside	C14H20O8
		Hydroxytyrosol 1-glycosides; 1-O-ß-D-Allopyranoside	C14H20O8
		Hydroxytyrosol 1-glycosides; 1-O-ß-D-Glucopyranoside	C14H20O8
		Laughine	C11H18BrN5O
		Lindbladiapyrone	C16H12O7
		2-Methyl-1,3,5-benzenetriol; 1-Me ether, 5-O-ß-D-glucopyranoside	C14H20O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
317.0958 (continued)	**700**	Orotidine; 3N-Me, Me ester	C12H16N2O8
		Pannaric acid	C16H12O7
		1,2,3,5,8-Pentahydroxyanthraquinone; 1,3-Di-Me ether	C16H12O7
		1,2,3,7,8-Pentahydroxyanthraquinone; 2,3-Di-Me ether	C16H12O7
		3',4,4',5',6-Pentahydroxyaurone; 4-Me ether	C16H12O7
		1,2,3,8,9-Pentahydroxycoumestan	C15H8O8
		2',3',5,6,7-Pentahydroxyflavanone; (S)-form, 6,7-Methylene ether	C16H12O7
		2',3,4',5,7-Pentahydroxyflavone; 2'-Me ether	C16H12O7
		2',3,4',5,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		2',3,4',5,7-Pentahydroxyflavone; 5-Me ether	C16H12O7
		2',3',4',6,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		2',3,5,7,8-Pentahydroxyflavone; 7-Me ether	C16H12O7
		2',4',5,6,7-Pentahydroxyflavone; 6-Me ether	C16H12O7
		2',5,6',7,8-Pentahydroxyflavone; 8-Me ether	C16H12O7
		3,3',4',6,7-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,3',4',6,7-Pentahydroxyflavone; 7-Me ether	C16H12O7
		3,3',4',7,8-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,3',4',7,8-Pentahydroxyflavone; 3'-Me ether	C16H12O7
		3,3',4',7,8-Pentahydroxyflavone; 8-Me ether	C16H12O7
		3',4',5,5',7-Pentahydroxyflavone; 3'-Me ether	C16H12O7
		3',4',5,5',7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3,4',5,6,7-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,4',5,6,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyflavone; 5-Me ether	C16H12O7
		3,4',5,6,7-Pentahydroxyflavone; 7-Me ether	C16H12O7
		3,4',5,7,8-Pentahydroxyflavone; 3-Me ether	C16H12O7
		3,4',5,7,8-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		3,4',5,7,8-Pentahydroxyflavone; 7-Me ether	C16H12O7
		3',4',5,7,8-Pentahydroxyflavone; 8-Me ether	C16H12O7
		3,5,6,7,8-Pentahydroxyflavone; 3-Me ether	C16H12O7
		4',5,6,7,8-Pentahydroxyflavone; 4'-Me ether	C16H12O7
		4',5,6,7,8-Pentahydroxyflavone; 6-Me ether	C16H12O7
		2',4',5,5',7-Pentahydroxyisoflavone; 5'-Me ether	C16H12O7
		3',4',5,5',7-Pentahydroxyisoflavone; 4'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyisoflavone; 3'-Me ether	C16H12O7
		3',4',5,6,7-Pentahydroxyisoflavone; 6-Me ether	C16H12O7
		3',4',5,7,8-Pentahydroxyisoflavone; 8-Me ether	C16H12O7
		4',5,6,7,8-Pentahydroxyisoflavone; 8-Me ether	C16H12O7
		1,2,3,4,5-Pentahydroxy-7-methylanthraquinone; 2-Me ether	C16H12O7
		1,2,3,7,8-Pentahydroxy-6-methylanthraquinone; 1-Me ether	C16H12O7
		1,2,3,7,8-Pentahydroxy-6-methylanthraquinone; 2-Me ether	C16H12O7
		1,3,4,5,7-Pentahydroxy-2-methylanthraquinone; 7-Me ether	C16H12O7

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
317.0958	**700**	1,3,5,6,8-Pentahydroxy-2-methylanthraquinone; 6-Me ether	C16H12O7
(continued)		1,3,5,7,8-Pentahydroxy-2-methylanthraquinone; 7-Me ether	C16H12O7
		3',4,4',5',6-Pentahydroxy-5-methylaurone	C16H12O7
		3,3',4',5,7-Pentahydroxy-6-methylflavone	C16H12O7
		3,3',4',5,7-Pentahydroxy-8-methylflavone	C16H12O7
		3,4',5,6,7-Pentahydroxy-8-methylflavone	C16H12O7
		3',4',5,6,7-Pentahydroxy-8-methylflavone	C16H12O7
		3,4',5,7,8-Pentahydroxy-6-methylflavone	C16H12O7
		3',4',5,5',7-Pentahydroxy-6-methylisoflavone	C16H12O7
		3,6a,7,8,9-Pentahydroxypterocarpan; 8,9-Methylene ether	C16H12O7
		proto-Quercitol; L-form, 1-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		scyllo-Quercitol, 9CI, 8CI; 2-O-(3,4,5-Trihydroxybenzoyl)	C13H16O9
		3,3a,7,7a-Tetrahydro-6-hydroxy-2(6H)-benzofuranone; (3aS,6R,7aR)-form, O-ß-D-Glucopyranoside	C14H20O8
		1,3,5,6-Tetrahydroxy-2-hydroxymethylanthraquinone; 6-Me ether	C16H12O7
		3',4',5,7-Tetrahydroxy-3-hydroxymethylflavone	C16H12O7
		3,3',4',5-Tetrahydroxy-7-methoxyflavone	C16H12O7
		3,3',4',7-Tetrahydroxy-5-methoxyflavone	C16H12O7
		3,3',5,7-Tetrahydroxy-4'-methoxyflavone	C16H12O7
		3',4',5,6-Tetrahydroxy-7-methoxyflavone	C16H12O7
		3,4',5,7-Tetrahydroxy-3'-methoxyflavone	C16H12O7
		3',4',5,7-Tetrahydroxy-3-methoxyflavone	C16H12O7
		3,4',5,7-Tetrahydroxy-6-methoxyflavone	C16H12O7
		3',4',5,7-Tetrahydroxy-6-methoxyflavone	C16H12O7
		3,4',5,7-Tetrahydroxy-8-methoxyflavone	C16H12O7
		3,4',6,7-Tetrahydroxy-5-methoxyflavone	C16H12O7
		3',5,7,8-Tetrahydroxy-4'-methoxyflavone	C16H12O7
		4',5,6,7-Tetrahydroxy-3'-methoxyflavone	C16H12O7
		4',5,7,8-Tetrahydroxy-3'-methoxyflavone	C16H12O7
		4,9,11,12a-Tetrahydroxyrotenoid	C16H12O7
		3,4,5-Trihydroxybenzaldehyde, 9CI; 4-O-ß-D-Glucopyranoside	C13H16O9
		3,4,5-Trihydroxybenzoic acid; a-L-Rhamnopyranosyl ester	C13H16O9
		2,5,7-Trihydroxy-4H-1-benzopyran-4-one; 2-O-(3-Hydroxy-4-methoxyphenyl)	C16H12O7
		5,6,7-Trihydroxy-2-(4-hydroxyphenoxy)-4H-1-benzopyran-4-one; 6-Me ether	C16H12O7
		5,7,10-Trihydroxy-3-methyl-1H-naphtho[2,3-c]pyran-1,6,9-trione; 5,7-Di-Me ether	C16H12O7
		2,5,8-Trihydroxy-6-methylxanthone-1-carboxylic acid; 8-Me ether	C16H12O7
		2,3,7-Trihydroxy-1,6-phenazinedicarboxylic acid, 9CI	C14H8N2O7
		Uridine-5-acetic acid; Me ester	C12H16N2O8
		Verrucarin G	C15H12N2O6

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137	**250**		
		2-Acetyl-1,6,8-trihydroxy-3-methylanthraquinone, 8CI; 8-O-ß-D-Glucoside	C23H22O11
		Acremolactone A	C26H34O8
		Agrimophol	C26H34O8
		Amoorastatin; O3-De-Ac	C26H34O8
		1,5-Anhydrofructose, 9CI; D-form, Tribenzoyl	C27H22O8
		1,6-Anhydromannose; ß-D-Pyranose-form, 2,3,4-Tribenzoyl	C27H22O8
		Apigenin 7-glycosides; 7-O-ß-D-Glucuronopyranoside, Et ester	C23H22O11
		Austalide B	C26H34O8
		Bacillisporin B	C26H18O9
		Bergenin; 11-O-(E-4-Hydroxycinnamoyl)	C23H22O11
		Bipinnatin B	C24H26O10
		Bisinaiquinone	C30H18O6
		Chicoric acid	C22H18O12
		Chicoric acid; (2R,3R)-form	C22H18O12
		Chicoric acid; (2S,3S)-form	C22H18O12
		Chicoric acid; (2RS,3RS)-form	C22H18O12
		Chicoric acid; (2RS,3SR)-form	C22H18O12
		15-Chloro-1,4-epoxy-5,8,9-trihydroxy-11(13)-germacren-12,6-olid-14-oic acid; (1ß,4ß,5ß,6a,8ß,9a,10ßH)-form, 8-(2-Hydroxymethyl-2-butenoyl), Me ester	C21H27ClO10
		Clausenolide; 1-Me ether	C26H34O8
		Cosmosiin; 2"-Ac	C23H22O11
		Cosmosiin; 3"-Ac	C23H22O11
		Cosmosiin; 4"-Ac	C23H22O11
		Cosmosiin; 6"-Ac	C23H22O11
		Cryptochlorophaeic acid; O-De-Me, 4,4'-di-Me ether	C26H34O8
		Cryptochlorophaeic acid; 4-Me ether	C26H34O8
		Cryptochlorophaeic acid; 4'-Me ether	C26H34O8
		Curcapitoside	C23H22O11
		1-[5-ß-D-Glucopyranosyl-4',5'-dihydro-3',4,4',6-tetrahydroxy-5'-(hydroxymethyl)spiro[benzofuran-2(3H),2'(3'H)-furan]-7-yl]ethanone, 9CI	C20H26O13
		Deacyltaiwanschirin; 10-Ac	C24H26O10
		4-N-Demethyl-4-N-ethyloxytetracycline	C23H26N2O9
		1-Deoxy-ß-D-fructopyranosyl-(1?2)-[1-deoxy-ß-D-fructopyranosyl-(1?3)]-L-arabinose	C17H30O15
		1-Deoxy-ß-D-fructopyranosyl-(1?2)-[1-deoxy-ß-D-fructopyranosyl-(1?3)]-L-arabinose; a-Furanose-form	C17H30O15
		6-Deoxymannonic acid, 9CI, 8CI; L-form, 1,5-Lactone, tribenzoyl	C27H22O8
		Derrisin; 11,12a-Dihydroxy, 4'-Me ether	C24H26O10
		a-D-Arabinofuranosyl-(1?2)-a-D-mannopyranosyl-(1?2)-D-glucose, 9CI	C17H30O15

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137	**250**	a-D-Arabinofuranosyl-(1?2)-a-D-mannopyranosyl-(1?2)-D-glucose, 9CI; a-Pyranose-form	C17H30O15
(continued)		ß-D-Arabinopyranosyl-(1?2)-a-D-mannopyranosyl-(1?2)-D-glucose	C17H30O15
		ß-D-Arabinopyranosyl-(1?2)-a-D-mannopyranosyl-(1?2)-D-glucose; a-Pyranose-form	C17H30O15
		D-Galactopyranosyl-(1?3)-D-galactopyranosyl-(1?3)-L-arabinose, 9CI	C17H30O15
		ß-D-Galactopyranosyl-(1?6)-ß-D-galactopyranosyl-(1?3)-L-arabinose, 8CI	C17H30O15
		ß-D-Glucopyranosyl-(1?2)-[ß-D-apiofuranosyl(1?6)]-D-glucose	C17H30O15
		ß-D-Glucopyranosyl-(1?2)-[ß-D-apiofuranosyl(1?6)]-D-glucose; ß-D-Pyranose-form	C17H30O15
		ß-D-Glucopyranosyl-(1?3)-ß-D-glucopyranosyl-(1?2)-D-xylose, 9CI	C17H30O15
		ß-D-Glucopyranosyl-(1?3)-ß-D-glucopyranosyl-(1?2)-D-xylose, 9CI; Pyranose-form	C17H30O15
		ß-D-Glucopyranosyl-(1?2)-[ß-D-glucopyranosyl-(1?4)]-L-arabinose, 8CI	C17H30O15
		a-D-Xylopyranosyl-(1?6)-ß-D-glucopyranosyl-(1?4)-D-glucose, 9CI	C17H30O15
		ß-D-Xylopyranosyl-(1?6)-ß-D-glucopyranosyl-(1?4)-D-glucose, 9CI	C17H30O15
		3,6:7,8-Diepoxy-13-hydroxy-18-oxo-3,5,11,15-cembratetraen-20,10-olide; (1R,7S,8S,10S,13R)-form, 17-Acetoxy, 11a,12a-epoxide, 13-Ac	C24H26O10
		14,15:19,29-Diepoxy-1,3,7,29-tetrahydroxy-11-meliacanone	C26H34O8
		14,15:19,29-Diepoxy-1,3,7,29-tetrahydroxy-11-meliacanone; (1a,3a,7a,14ß,15ß,29R)-form	C26H34O8
		7,8:17,18-Diepoxy-1(19),6(20),10(17),13-xenicatetraene-11,12,18-triol; Tri-Ac	C26H34O8
		Dihydro-3-(hydroxymethyl)-4-[bis(3,4,5-trihydroxyphenyl)methyl]-2(3H)furanone; (8R*,8'R*)-form, 3,4-Methylene, 3',5,5'-tri-Me ether, 7-Ac	C24H26O10
		3',4'-Dihydroxyacetophenone, 8CI; 3'-Me ether, 4'-O-[a-L-rhamnopyranosyl-(1?6)-ß-D-glucopyranoside]	C21H30O12
		3',4'-Dihydroxyacetophenone, 8CI; 3'-Me ether, 4'-O-[4-hydroxy-E-cinnamoyl-(?6)-ß-D-glucopyranoside]	C24H26O10
		6,7-Dihydroxy-2H-1-benzopyran-2-one, 9CI; 7-O-[4-Hydroxyphenylacetyl-(?6)-ß-D-glucopyranoside]	C23H22O11
		1-(3,4-Dihydroxybenzoyl)-3,6,7-naphthalenetriol; 3-O-ß-D-Glucopyranoside	C23H22O11
		6-O-(3,4-Dihydroxycinnamoyl)glucose; ß-D-Pyranose-form, 2,3,4-Trihydroxy-3-methylbutyl glycoside, 3'-Me ether	C21H30O12

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137	**250**	4,8-Dihydroxy-2,10(14),11(13)-guaiatrien-12,6-olide; (1a,4a,5a,6a,8ß)-form, 8-O-[4-Hydroxy-2-(2-hydroxymethyl-2E-butenoyloxy)methyl-2E-butenoyl]	C25H30O9
(continued)		5,7-Dihydroxy-1(3H)-isobenzofuranone, 9CI; 5-Me ether, 7-O-[ß-D-xylopyranosyl-(1?6)-ß-D-glucopyranoside]	C20H26O13
		4,13-Dihydroxylupanine; (4?,13?)-form, O13-(3,4,5-Trimethoxybenzoyl)	C25H34N2O7
		5,7-Dihydroxy-4'-methoxyflavone, 8CI; 7-O-ß-D-Glucuronopyranoside, Me ester	C23H22O11
		4',5-Dihydroxy-6,7-methylenedioxyisoflavone; 5-Me ether, 4'-O-ß-D-glucopyranoside	C23H22O11
		2,3-Dihydroxy-2-(2-methylpropyl)butanedioic acid; (2R,3S)-form, 1-(4-ß-D-Glucopyranosyloxybenzyl) ester	C21H30O12
		2,3-Dihydroxy-2-(2-methylpropyl)butanedioic acid; (2R,3S)-form, 4-(4-ß-D-Glucopyranosyloxybenzyl) ester	C21H30O12
		1-(3,4-Dihydroxyphenyl)-1-propanone, 9CI; 3-Me ether, 4-O-[ß-D-apiofuranosyl-(1?6)-ß-D-glucopyranoside]	C21H30O12
		3-(3,4-Dihydroxyphenyl)-2-propenoic acid, 9CI; (E)-form, [ß-D-Apiofuranosyl-(1?6)-ß-D-glucopyranosyl] ester	C20H26O13
		3-(3,4-Dihydroxyphenyl)-2-propen-1-ol; (E)-form, 3'-Me ether, 1-O-[ß-D-apiofuranosyl-(1?6)-ß-D-glucopyranoside]	C21H30O12
		3-(3,4-Dihydroxyphenyl)-2-propen-1-ol; (E)-form, 3'-Me ether, 4'-O-[ß-D-apiofuranosyl-(1?2)-ß-D-glucopyranoside]	C21H30O12
		3-(3,4-Dihydroxyphenyl)-2-propen-1-ol; (E)-form, 3'-Me ether, 1-O-[a-L-arabinofuranosyl-(1?6)-ß-D-glucopyranoside]	C21H30O12
		6-(3,7-Dimethyl-2,6-octadienyl)-5,7-dihydroxy-8-(2-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one	C30H34O5
		6-(3,7-Dimethyl-2,6-octadienyl)-5,7-dihydroxy-8-(2-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one; (?)-(E)-form	C30H34O5
		6-(3,7-Dimethyl-2,6-octadienyl)-5,7-dihydroxy-8-(2-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one; (?)-(E)-form, 3-Methylbutanoyl isomer	C30H34O5
		8-(3,7-Dimethyl-2,6-octadienyl)-5,7-dihydroxy-6-(2-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one	C30H34O5
		8-(3,7-Dimethyl-2,6-octadienyl)-5,7-dihydroxy-6-(2-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one; (?)-(E)-form	C30H34O5
		8-(3,7-Dimethyl-2,6-octadienyl)-5,7-dihydroxy-6-(2-methyl-1-oxobutyl)-4-phenyl-2H-1-benzopyran-2-one; (?)-(E)-form, 3-Methylbutanoyl isomer	C30H34O5

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137 (continued)	**250**	1,16-Eicosanediol; (?)-form, Di-O-sulfate	C20H42O8S2
		Epiphorellic acid 1	C26H34O8
		15,16-Epoxy-1,2-dihydroxy-3,13(16),14-clerodatrien-17,12-olid-18-oic acid; (ent-1ß,2ß,12ßH)-form, Di-Ac, Me ester	C25H30O9
		10,14-Epoxy-2,8-dihydroxy-3,11(13)-guaiadien-12,6-olide; (1a,2ß,5a,6a,8ß,10a)-form, 8-[2-(2-Hydroxymethyl-2E-butenoyloxymethyl)-2E-butenoyl]	C25H30O9
		1,4-Epoxy-8,13-dihydroxy-3-oxo-1,5,7(11)-germacratrien-12,6-olide; (4a,5E,8a,10ß)-form, 8-(4-Acetoxy-3-methyl-2-butenoyl)(Z-), 13-Ac	C24H26O10
		3,4-Epoxy-8,9-dihydroxy-2-oxo-1(10),11(13)-guaiadien-12,6-olide; (3a,4a,5a,6a,8ß,9ß)-form, 8-(2-Acetoxymethyl-2E-butenoyl), 9-Ac	C24H26O10
		19,29-Epoxy-1,3,7,12,29-pentahydroxy-14-meliacen-11-one	C26H34O8
		19,29-Epoxy-1,3,7,12,29-pentahydroxy-14-meliacen-11-one; (1a,3a,7a,12a,29R)-form	C26H34O8
		14,15-Epoxy-3,5,16,19-tetrahydroxybufa-20,22-dienolide; (3ß,5ß,14ß,15ß,16ß)-form, 16-Ac	C26H34O8
		19,29-Epoxy-1,3,7,29-tetrahydroxymeliacane-11,15-dione	C26H34O8
		19,29-Epoxy-1,3,7,29-tetrahydroxymeliacane-11,15-dione; (1a,3a,7a,29R)-form	C26H34O8
		8,17-Epoxy-2,9,12-trihydroxy-5,13-briaradien-18,7-olide; (2ß,5Z,7a,8a,9ß,12a,17a)-form, 12-Ketone, 2-butanoyl, 9-Ac	C26H34O8
		Euchrenone a4	C30H34O5
		Euchrenone a4; (S)-form	C30H34O5
		Fleminginin	C30H34O5
		8-Galactopyranosyl-4',5,7-trihydroxyflavone; 6''-O-Ac	C23H22O11
		Gelliusine E; (±)-form, 5-Deoxy, 6-bromo	C20H20Br2N4
		8-Glucofuranosyl-4',5,7-trihydroxyflavone; 2''-Acetyl	C23H22O11
		3',4,4',5,5',9,9'-Heptahydroxy-2,7'-cyclolignan; (7'S,8R,8'R)-form, 3',5,5'-Tri-Me ether, 9,9'-di-Ac	C25H30O9
		3,3',4,4',5,9,9'-Heptahydroxylignan; (8R,8'R)-form, 3',4'-Methylene, 3,5-di-Me ether, 9,9'-di-Ac	C25H30O9
		1,3,5,11,14,19-Hexahydroxybufa-20,22-dienolide; (1ß,3ß,5ß,11a,14ß)-form, 1,3,5-Orthoacetate	C26H34O8
		3',4,4',5,9,9'-Hexahydroxy-2,7'-cyclolignan; (7'S,8R,8'R)-form, 3',5-Di-Me ether, 9-O-(5-hydroxy-4-oxopentanoyl)	C25H30O9
		3',4,4',5,9,9'-Hexahydroxy-2,7'-cyclolignan; (7'S,8R,8'R)-form, 3',5-Di-Me ether, 9'-O-(5-hydroxy-4-oxopentanoyl)	C25H30O9
		3,3',4,4',9,9'-Hexahydroxylignan; (8R,8'R)-form, 3,3',4,4'-Tetra-Me ether, 9,9'-di-Ac	C26H34O8
		4'-Hydroxyacetophenone, 8CI; O-[4-Hydroxy-3-methoxy-E-cinnamoyl-(?6)-ß-D-glucopyranoside]	C24H26O10

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137	**250**	8-Hydroxy-1(10),3,11(13)-guaiatrien-12,6-olide; (5a,6a,8ß)-form, [4-Hydroxy-2-(4-hydroxy-2-hydroxymethyl-2E-butenoyloxymethyl)-2E-butenoyl]	C25H30O9
(continued)		8-Hydroxy-3,10(14),11(13)-guaiatrien-12,6-olide; (1a,5a,6a,8ß)-form, 8-[4-Hydroxy-2-(4-hydroxy-2-hydroxymethyl-2-butenoyloxymethyl)-2-butenoyl](E,E-)	C25H30O9
		Hypholomin A	C26H18O9
		Isocryptochlorophaeic acid; 4-Me ether	C26H34O8
		4-Isopropylbenzyl alcohol; O-[ß-D-Glucopyranosyl-(1?2)-ß-D-galactopyranoside]	C22H34O11
		2-Isopropyl-5-methyl-1,4-benzenediol; 5-O-[a-L-Rhamnopyranosyl-(1?2)-ß-D-glucopyranoside]	C22H34O11
		2-Isopropyl-5-methylphenol; O-[ß-D-Glucopyranosyl-(1?2)-ß-D-glucopyranoside]	C22H34O11
		5-Isopropyl-2-methylphenol; O-[ß-D-Glucopyranosyl-(1?2)-ß-D-glucopyranoside]	C22H34O11
		Kadsulignan A; 6ß-Acetoxy	C25H30O9
		Kaempferol 3-glycosides; Monoglycosides, 3-O-(2-O-Acetyl-a-L-rhamnopyranoside)	C23H22O11
		Kaempferol 3-glycosides; Monoglycosides, 3-O-(3-O-Acetyl-a-L-rhamnopyranoside)	C23H22O11
		Kaempferol 3-glycosides; Monoglycosides, 3-O-(4-O-Acetyl-a-L-rhamnopyranoside)	C23H22O11
		Kopsaporine; 12-Methoxy, 14,15-dihydro, 15a-hydroxy	C24H30N2O8
		a-L-Arabinofuranosyl-(1?6)-[ß-D-galactopyranosyl-(1?3)]-D-galactose	C17H30O15
		a-L-Arabinofuranosyl-(1?6)-[a-D-glucopyranosyl-(1?4)]-D-glucose, 9CI	C17H30O15
		a-L-Arabinofuranosyl-(1?6)-[a-D-glucopyranosyl-(1?4)]-D-glucose, 9CI; ß-Pyranose-form	C17H30O15
		a-L-Arabinofuranosyl-(1?2)-[a-D-mannopyranosyl-(1?6)]-D-mannose	C17H30O15
		a-L-Arabinofuranosyl-(1?2)-[a-D-mannopyranosyl-(1?6)]-D-mannose; a-form	C17H30O15
		Ligulaverin C; 6-Deacyl, 6-angeloyl	C26H34O8
		Loxodinol	C25H30O9
		Luteusin C; Stereoisomer 1, Me ether	C26H31ClO6
		Mallotophilippen C	C30H34O5
		Maximowicziol A; 11-Ketone, 4-O-ß-D-glucopyranoside	C25H30O9
		8-Methoxy-3,4-methylenedioxy-1-phenanthrenecarboxylic acid; 6-Hydroxy, O-ß-D-glucopyranoside	C23H22O11
		5-Methyl-2-hexene-1,4,5-triol; 4-(4-Hydroxybenzoyl), 1,5-dibenzoyl	C28H26O7
		Millewanin D	C30H34O5
		Munronin F	C25H30O9
		Murrafoline B	C32H30N2O2
		Murrafoline B; (±)-form	C32H30N2O2
		Murrafoline D	C32H30N2O2
		Murrafoline D; (±)-form	C32H30N2O2

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137 (continued)	**250**	Murrafoline G	C32H30N2O2
		Murrafoline G; (±)-form	C32H30N2O2
		Murrastifoline D; 3'-Deoxy	C32H30N2O2
		Neocalyxin A	C28H26O7
		Neocalyxin A; 2-Epimer	C28H26O7
		Nordracorubin	C31H22O5
		Nordracorubin; (S)-form	C31H22O5
		Nordracorubin; (±)-form	C31H22O5
		Nostocyclamide	C20H22N6O4S2
		3,3',4,4',5,5',9,9'-Octahydroxy-7,7'-epoxylignan; (7S,7'S,8R,8'R)-form, 3,4:3',4'-Bis(methylene), 5,5'-di-Me ether, 9-Ac	C24H26O10
		3,3',4,4',5,5',9,9'-Octahydroxy-7,7'-epoxylignan; (7R,7'S,8R,8'R)-form, 3,4:3',4'-Bis(methylene), 5,5'-di-Me ether, 9-Ac	C24H26O10
		3,3',4,4',5,5',9,9'-Octahydroxy-7,7'-epoxylignan; (7S,7'R,8R,8'R)-form, 3,4:3',4'-Bis(methylene), 5,5'-di-Me ether, 9-Ac	C24H26O10
		Oganomycin GI	C16H18N4O7S3
		1,3,5,14,19-Pentahydroxybufa-20,22-dienolide; (1ß,3ß,5ß,14ß)-form, 19-Aldehyde, 3-Ac	C26H34O8
		3,6,8,12,14-Pentahydroxybufa-4,20,22-trienolide; (3ß,6ß,12ß,14ß)-form, 6-Ac	C26H34O8
		3,4',5,6,7-Pentahydroxyflavone; 6,7-Methylene, 4',5-di-Me ether, 3-O-ß-D-xylopyranoside	C23H22O11
		3',4',5',6,7-Pentahydroxyisoflavone; 3',4'-Methylene, 5'-Me ether, 6-O-a-L-rhamnopyranoside	C23H22O11
		3',4',5',6,7-Pentahydroxyisoflavone; 3',4'-Methylene, 7-Me ether, 6-O-a-L-rhamnopyranoside	C23H22O11
		1,3,7,11,12-Pentahydroxy-14-meliacen-28-oic acid; (1a,3a,7a,11ß,12a)-form, 7-Ketone	C26H34O8
		Perophoramidine	C21H17BrCl2N4
		Petromurin C; 5"-Methoxy	C27H26N2O6
		Phelligridin E	C25H14O10
		1,18-Phytanediol; Disulfate	C20H42O8S2
		Picrasin A	C26H34O8
		Podophyllic acid; (7R,7'R,8R,8'R)-form, N-Ethylhydrazide	C24H30N2O8
		Poinsettifolin B	C30H34O5
		4-(2-Propenyl)-1,2-benzenediol, 9CI; Di-O-ß-D-glucopyranoside	C21H30O12
		5-(2-Propenyl)-1,2,3-benzenetriol; 1-Me ether, 3-O-[ß-D-apiofuranosyl-(1?6)-ß-D-glucopyranoside]	C21H30O12
		5-(2-Propenyl)-1,2,3-benzenetriol; 1-Me ether, 3-O-[a-L-arabinopyranosyl-(1?6)-ß-D-glucopyranoside]	C21H30O12
		Protocetraric acid; 1'-O-(3-Carboxypropanoyl)	C22H18O12
		Puerol A; 4"-Me ether, 2"-O-ß-D-glucopyranoside	C24H26O10
		Punaglandin 1; Bromo analogue, tri-O-de-Ac	C21H31BrO7
		Rhinacanthin Q	C28H26O7

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137 (continued)	**250**	Robustaol A	C25H30O9
		Rubraflavone A; 8-(3-Methyl-2-butenyl)	C30H34O5
		Saroaspidin C	C26H34O8
		Scandoside; 6-Butanoyl, Me ester	C21H30O12
		Scandoside; 6-Epimer, 6-butanoyl, Me ester	C21H30O12
		4,15-Scirpenediol; 4ß-form, Dibenzoyl	C29H30O6
		Sclerotiorin; (7S,11E)-form, O-De-Ac, O-(4-carboxy-3-methyl-2E-butenoyl)	C25H27ClO7
		Sclerotiorin; (7S,11E)-form, O-De-Ac, O-(4-carboxy-3-methyl-2Z-butenoyl)	C25H27ClO7
		Sclerotiorin; (7S,11E)-form, O-De-Ac, O-(4-carboxy-3-methyl-3E-butenoyl)	C25H27ClO7
		Sclerotiorin; (7S,11E)-form, O-De-Ac, O-(4-carboxy-3-methyl-3Z-butenoyl)	C25H27ClO7
		Spinonin; 4'-Me ether	C24H26O10
		13(16),14-Spongiadiene-2,3,17,19-tetrol; 3a-form, 2-Ketone, tri-Ac	C26H34O8
		Terretonin D	C26H34O8
		2,4,6,8,10,12-Tetradecahexaenedioic acid; (all-E)-form, 1-L-Aspartic acid, 14-L-isoleucine diamide	C24H30N2O8
		5,6,8,13-Tetrahydro-1,7,9,11-tetrahydroxy-8,13-dioxo-3-(2-oxopropyl)benzo[a]naphthacene-2-carboxylic acid, 9CI	C26H18O9
		3',4,4',5-Tetrahydroxy-2,7'-cyclolign-7-ene-9,9'-dioic acid; (7'R,8'S)-form, 9-O-(1S,2-Dicarboxyethyl) ester	C22H18O12
		1,4,6,9-Tetrahydroxydihydro-ß-agarofuran; (1a,4ß,6ß,9ß)-form, 1-Benzoyl, 6,9-di-Ac	C26H34O8
		1,6,8,9-Tetrahydroxydihydro-ß-agarofuran; (1a,6ß,8a,9a)-form, 9-Benzoyl, 1,6-di-Ac	C26H34O8
		3,3',4',7-Tetrahydroxyflavone; 3',4'-Methylene, 3-Me ether, 7-O-ß-D-glucopyranoside	C23H22O11
		2',4',5',7-Tetrahydroxyisoflavone; 4',5'-Methylene, 2'-Me ether, 7-O-ß-D-glucopyranoside	C23H22O11
		2',5,6,7-Tetrahydroxyisoflavone; 6,7-Methylene, 5-Me ether, 2'-O-ß-D-glucopyranoside	C23H22O11
		3',4',6,7-Tetrahydroxyisoflavone; 3',4'-Methylene, 6-Me ether, 7-O-ß-D-glucopyranoside	C23H22O11
		3,5,11,14-Tetrahydroxy-12-oxobufa-20,22-dienolide; (3ß,5ß,11a,14ß)-form, 3-Ac	C26H34O8
		3,5,12,14-Tetrahydroxy-11-oxobufa-20,22-dienolide; (3ß,5ß,12ß,14ß)-form, 3-Ac	C26H34O8
		O,O,O',O'-Tetrapropyl 2,2'-(1,2-dimethyl-1,2-ethanediylidene)bis(phosphorohydrazidothioate)	C16H36N4O4P2S2
		m-Trigallic acid	C21H14O13
		p-Trigallic acid	C21H14O13
		2,3,14-Trihydroxy-5,8(17),11-briaratrien-18,7-olide; (2ß,3a,5Z,7a,14a)-form, Tri-Ac	C26H34O8

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
475.137	**250**	6,10,14-Trihydroxy-3,7,11,15(17)-cembratetraen-16,2-olide; (1S,2S,3E,6S,7E,10S,11E,14S)-form, Tri-Ac	C26H34O8
(continued)		5,7,8-Trihydroxyflavone; 5,8-Di-Me ether, 7-O-ß-D-glucuronoside	C23H22O11
		2,4,5-Trihydroxy-2-(hydroxymethyl)pentanoic acid; (2R,4S)-form, 1,4-Lactone, tribenzoyl	C27H22O8
		4',5,7-Trihydroxyisoflavone; 7-O-(6-O-Acetyl-ß-D-glucopyranoside)	C23H22O11
		1,3,6-Trihydroxy-2-methylanthraquinone; 3-O-(6-O-Acetyl-ß-D-glucopyranoside)	C23H22O11
		1,3,8-Trihydroxy-6-methylanthraquinone, 8CI; 1-O-(6-O-Acetyl-ß-D-glucopyranoside)	C23H22O11
		5,6,8-Trihydroxy-28-norisotoonafolin	C25H30O9
		5,6,8-Trihydroxy-28-norisotoonafolin; (5a,6ß,8a)-form	C25H30O9
		2,11,12-Trihydroxy-6,7-seco-8,11,13-abietatriene-6,7-dial 11,6-hemiacetal; 2a-form, Tri-Ac(6a-)	C26H34O8
		4',5,7-Trihydroxy-3',5',8-triprenylisoflavone	C30H34O5
		4',5,7-Trihydroxy-3',6,8-triprenylisoflavone	C30H34O5
		Trillenogenin; 1-Ketone	C26H34O8
		Tryptoquivaline G; Ac	C25H22N4O6
		Tuliposide E	C21H30O12
		Vaccariose	C17H30O15
		Vitexin; 2"-Ac	C23H22O11
		Vitexin; 3"-Ac	C23H22O11
		Vitexin; 6"-Ac	C23H22O11
		14(17)-Vouacapene-1,5,6,7-tetrol; (1a,5a,6a,7ß)-form, 1,6,7-Tri-Ac	C26H34O8
		Xenione	C26H34O8
		Xenione; Stereoisomer	C26H34O8
513.0843	**700**		
		Agaricoglyceride A; 2-O-Deacyl, 2-Ac	C19H14Cl4O8
		Aspergillic acid, 8CI; (S)-form, Zn complex	C24H38N4O4Zn
		Daphmacrine	C32H49NO4
		Daphnioldhanin F; (-)-form, 25-Ketone, 26-Ac	C32H49NO4
		Diphlorethohydroxycarmalol	C24H16O13
		1-Galloylglucose; ß-D-Pyranose-form, 6-O-(4-Hydroxy-3,5-dimethoxybenzoyl)	C22H24O14
		5-(Hydroxymethyl)-1,2,3-benzenetriol; 1'-O-(3,4,5-Trihydroxybenzoyl), 2-O-(6-O-acetyl-ß-D-glucopyranoside)	C22H24O14
		2-Hydroxymethyl-1,4,5,7-tetrahydroxyanthraquinone; Penta-Ac	C25H20O12
		2',3,4',5,7-Pentahydroxyflavone; Penta-Ac	C25H20O12
		3',4',5,5',7-Pentahydroxyflavone; Penta-Ac	C25H20O12
		3,4',5,7,8-Pentahydroxyflavone; Penta-Ac	C25H20O12
		3,4',6,7,8-Pentahydroxyflavone; Penta-Ac	C25H20O12
		Sarain A	C32H51N2O3
		Stellettazole B	C30H51N6O
		Tauroacidin A; 9-Deoxy	C13H16Br2N6O4S

Peak m/z	Searched Range (ppm)	Names of Putative Compounds	Chemical Formula
513.0843 (continued)	**700**	3,4,5-Trihydroxybenzoic acid; 3-Me ether, 4-O-[3,4-dihydroxy-5-methoxybenzoyl-(?6)-ß-D-glucopyranoside]	C22H24O14

Table A.3. Compounds and corresponding molecular formulae identified as putative matches for Compound X as identified by FTMS, assuming molecular ions were potassium adducts.

Peak m/z[a]	m/z - K+	Low limit 10 ppm[b] range (molar mass)	High limit 10 ppm range (molar mass)	# of library matches
257.0300723	218.0669053	218.0647246	218.069086	0
317.0957864	278.1326194	278.1298381	278.1354007	8

Compound Name	Molecular Formula
4,4'-Dihydroxy-2,7'-cycloligna-7,7'-diene; 4'-Me ether	C19H18O2
1,7-Diphenyl-3,5-heptanediol; (3S,5S)-form, 1,2:6,7-Tetradehydro(E,E-), 3-ketone	C19H18O2
7,16-Epoxy-20-nor-5(10),6,8,11,13-cleistanthapentaen-3-one; 1,2-Didehydro	C19H18O2
Eupomatenoid 6; Me ether	C19H18O2
Lewisone	C19H18O2
Pantetheine	C11H22N2O4S
Pantetheine; (R)-form	C11H22N2O4S
3',4',5,7-Tetramethylflavone	C19H18O2

475.1369619	436.1737949	436.1694332	436.1781566	32[c]

Compound Name	Molecular Formula
Bruceolide; 15-Deoxy, parent acid, Et ester	C22H28O9
4,18:15,16-Diepoxy-6,8,10,19-tetrahydroxy-13(16),14-clerodadien-20,12-olide; (ent-4ß,6ß,8a,10a,12aH)-form, 19-Ac	C22H28O9
4,18:15,16-Diepoxy-6,8,10,19-tetrahydroxy-13(16),14-clerodadien-20,12-olide; (ent-4ß,6ß,8a,10a,12ßH)-form, 19-Ac	C22H28O9
1,10:4,5-Diepoxy-8,13,14-trihydroxy-2,7(11)-germacradien-12,6-olide; (1a,2Z,4a,5ß,6a,8a,10a)-form, 8-(2-Methylbutanoyl), 13-Ac	C22H28O9
6,8-Dihydroxy-15-oxo-1,3,11(13)-elematrien-12,9-olide; (5ßH,6a,8ß,9ß,10aMe)-form, 1R,2-Epoxide, 6-O-(2-hydroxy-2-methylbutanoyl), 8-Ac	C22H28O9
1,8-Dihydroxy-15-oxo-11(13)-eudesmen-12,6-olide; (1ß,4ß,6a,8a)-form, 8-(4-Acetoxy-2-hydroxymethyl-2E-butenoyl)	C22H28O9

Table A.3 continued.

Peak m/z[a]	m/z - K+	Low limit 10 ppm[b] range (molar mass)	High limit 10 ppm range (molar mass)	# of library matches
475.1369619	436.1737949	436.1694332	436.1781566	32
Compound Name			**Molecular Formula**	
1,8-Dihydroxy-15-oxo-11(13)-eudesmen-12,6-olide; (1ß,4ß,6a,8a)-form, 8-(4-Acetoxy-3-hydroxy-2-methylenebutanoyl)			C22H28O9	
1,8-Dihydroxy-15-oxo-11(13)-eudesmen-12,6-olide; (1ß,4a,6a,8a)-form, 8-(4-Acetoxy-3-hydroxy-2-methylenebutanoyl)			C22H28O9	
1-(3,4-Dihydroxyphenyl)-2-(3,5-dihydroxyphenyl)ethane; 3,4-Di-Me ether, 3'-O-ß-D-glucopyranoside			C22H28O9	
1-(3,5-Dihydroxyphenyl)-2-phenyl-1,2-ethanediol; (1?,2?)-form, 3',5'-Di-Me ether, 1-O-ß-D-glucopyranoside			C22H28O9	
1,10-Epoxy-3,8-dihydroxy-4,11(13)-germacradien-12,6-olide; (1ß,3ß,4Z,6a,8ß,10a)-form, 8-O-(4-Hydroxy-2-hydroxymethyl-2E-butenoyl), 3-Ac			C22H28O9	
1,4-Epoxy-1,8,10,13-tetrahydroxy-5,7(11)-germacradien-12,6-olide; (1a,4ß,8a,10a)-form, 1-Me ether, 8-O-(2-methylpropenoyl), 13-Ac			C22H28O9	
1,4-Epoxy-1,8,10,13-tetrahydroxy-5,7(11)-germacradien-12,6-olide; (1a,4ß,8a,10a)-form, 8-Tigloyl, 13-Ac			C22H28O9	
1,4-Epoxy-1,8,10,13-tetrahydroxy-5,7(11)-germacradien-12,6-olide; (1ß,4ß,8a,10a)-form, 8-Tigloyl, 13-Ac			C22H28O9	
1,4-Epoxy-1,8,13-trihydroxy-5,7(11)-germacradien-12,6-olide; (1a,4ß,5E,8ß,10ßH)-form, 8-O-(4-Hydroxy-2-methyl-2E-butenoyl), 13-Ac			C22H28O9	
14,15-Epoxy-3,9,14-trihydroxy-5(15)-isocedren-12,4-olide; (3ß,4ß,9a,14a)-form, 14-(3R*-Hydroxy-2R*-methylbutanoyl), 3-Ac			C22H28O9	

Table A.3 continued.

Peak m/z[a]	m/z - K+	Low limit 10 ppm[b] range (molar mass)	High limit 10 ppm range (molar mass)	# of library matches
475.1369619	436.1737949	436.1694332	436.1781566	32
Compound Name			**Molecular Formula**	
14,15-Epoxy-3,9,14-trihydroxy-5(15)-isocedren-12,4-olide; (3ß,4ß,9a,14a)-form, 14-(3S*-Hydroxy-2R*-methylbutanoyl), 3-Ac			C22H28O9	
14,15-Epoxy-3,9,14-trihydroxy-5(15)-isocedren-12,4-olide; (3ß,4ß,9a,14a)-form, 14-(3-Hydroxy-3-methylbutanoyl), 3-Ac			C22H28O9	
Glaucarubol; 1-Epimer, 2-ketone, 15-Ac			C22H28O9	
Glaucarubol; 2-Ketone, 15-Ac			C22H28O9	
3,3',4,4',5,5',9,9'-Octahydroxy-7,7'-epoxylignan; (7S,7'S,8R,8'R)-form, 3,3',5,5'-Tetra-Me ether			C22H28O9	
3,3',4',5,5',7',9,9'-Octahydroxy-4,8'-oxyneolign-7-ene; (7E,7'R*,8'S*)-form, 3,3',5,5'-Tetra-Me ether			C22H28O9	
Samaderine Z; 15-Ac			C22H28O9	
3,4,8,15-Tetrahydroxy-10(14),11(13)-guaiadien-12,6-olide; (1a,3ß,4aOH,5a,6a,8a)-form, 8-(4-Hydroxy-2-methyl-2-butenoyl), 15-Ac			C22H28O9	
Bruceolide; 15-Deoxy, parent acid, Et ester			C22H28O9	
4,18:15,16-Diepoxy-6,8,10,19-tetrahydroxy-13(16),14-clerodadien-20,12-olide; (ent-4ß,6ß,8a,10a,12aH)-form, 19-Ac			C22H28O9	
4,18:15,16-Diepoxy-6,8,10,19-tetrahydroxy-13(16),14-clerodadien-20,12-olide; (ent-4ß,6ß,8a,10a,12ßH)-form, 19-Ac			C22H28O9	
1,10:4,5-Diepoxy-8,13,14-trihydroxy-2,7(11)-germacradien-12,6-olide; (1a,2Z,4a,5ß,6a,8a,10a)-form, 8-(2-Methylbutanoyl), 13-Ac			C22H28O9	
6,8-Dihydroxy-15-oxo-1,3,11(13)-elematrien-12,9-olide; (5ßH,6a,8ß,9ß,10aMe)-form, 1R,2-Epoxide, 6-O-(2-hydroxy-2-methylbutanoyl), 8-Ac			C22H28O9	
1,8-Dihydroxy-15-oxo-11(13)-eudesmen-12,6-olide; (1ß,4ß,6a,8a)-form, 8-(4-Acetoxy-2-hydroxymethyl-2E-butenoyl)			C22H28O9	

Table A.3 continued.

Peak m/z[a]	m/z - K+	Low limit 10 ppm[b] range (molar mass)	High limit 10 ppm range (molar mass)	# of library matches
475.1369619	436.1737949	436.1694332	436.1781566	32
Compound Name			**Molecular Formula**	
1,8-Dihydroxy-15-oxo-11(13)-eudesmen-12,6-olide; (1ß,4ß,6a,8a)-form, 8-(4-Acetoxy-3-hydroxy-2-methylenebutanoyl)			C22H28O9	
Wybutine; (S)-form, 3-ß-D-Ribofuranosyl			C18H24N6O7	
513.084279	474.121112	474.1163708	474.1258532	0
528.0473004	489.0841334	489.0792426	489.0890242	0
565.2863106	526.3231436	526.3178804	526.3284068	1
Compound Name			**Molecular Formula**	
Tryptophyllin L 2.1			C28H42N6O4	

[a]m/z = mass to charge ratio of molecular ion fragments identified by the mass spectrometer
[b]ppm = parts per million
[c]with the exception of Wybutine, all of these matched at 2 ppm